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RESEARCH ARTICLE

Balanced amino acid and higher micronutrients in millets complements legumes for improved human dietary nutrition

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Abstract

Background and objectives: More than 2 billion people suffer with malnutrition arising from dietary protein and micronutrients deficiencies. To enhance the dietary nutrient quality, the current study used two largely grown varieties of finger millet, pearl millet, pigeonpea, and chickpea to evaluate the effect of millet–legume blends for their enhanced protein digestibility, amino acid profiles, and essential micronutrients.

Findings: Our study revealed the presence of significant levels of proteins (6.3%–22.3%), essential amino acids, and micronutrients (Fe: 2.6–8.5 mg; Zn: 2–5.5 mg; Ca: 22-450 mg in 100 g) in these varieties. When specific millets combined with legumes in 3:1 proportion, significantly enhanced nutritional value of food by providing a balanced amino acid with good protein digestibility, and high levels of iron (7.58 mg) and zinc (4.96 mg) with 100 g of pearl millet and calcium (400.57 mg) with 100 g of finger millet.

Conclusions: Pigeonpea and chickpea have a good level of proteins with essential amino acids except methionine and cysteine, whereas millet had balanced amino acid including methionine and cysteine (50% higher) and much higher levels of micronutrients (Fe, Zn and Ca). Therefore, specific millets and legumes combination complemented higher levels of micronutrients in addition to complete proteins to support comprehensive human nutrition.

Significance and novelty: This study opens prospects for selecting complementary nutrient-dense varieties for household consumption. Industries can explore these product developments significantly to reduce malnutrition if consumed adequately, which is not possible with polished rice, refined wheat flour or maize even if it is combined with legumes.

KEYWORDS
daily value, in vitro protein digestibility, millet–legume combination, millets, protein and micronutrients

1 | INTRODUCTION

As vegetarian and vegan diets are on the rise in the West and protein and micronutrient deficiencies remain high across Asia and Africa, it is imperative to find sources of high quality and quantity of protein and major micronutrients in plant-based diet. The three most commonly eaten grains—polished rice, maize, and refined wheat cannot provide complete protein as they are extremely low in all the essential amino acids. Even when combined with legumes that are known to be good sources of protein, they still do not provide complete protein as legumes have low levels of methionine and cysteine amino acid and varying levels of protein digestibility. However, when legumes are combined with millets, they can provide complete protein with high digestibility and a wider range of micronutrients.

Good nutrition is a key to human growth, health, and functioning (Branca, Piwoz, Schultink, & Sullivan, 2015). Staple cereals and legumes are supposed to provide significant levels of protein, vitamins, and essential minerals besides mounted carbohydrates. Millets and grain legumes are dryland crops that are fairly rich in these nutritional qualities, are resilient and largely grow in semi-arid and tropical regions. They are known to be the climate-smart crops and overall "Smart Food," that is, food that is good for you, good for the planet, and good for the farmers. However, they have been overshadowed and replaced by the Big three crops—rice, wheat, and maize—in many parts of Africa and Asia and have over time been given less attention in terms of investment, research, and support. Hence, their value chains are less developed and they are commonly used as fodder for animals.

On the other hand, protein malnutrition is widespread in developing countries. Animal protein too is inadequate, unaffordable and not sustainable (Nazim, Mitra, Rahman, Abdullah, & Parveen, 2013). So, people depend mostly on grain legumes for protein (Saxena, Kumar, & Sultana, 2010). In the recent past, significant research efforts have been made toward identifying other sources of protein from legumes (Nazim et al., 2013). However, the nutritional value and quality of protein in different sources depend on the composition of essential amino acids and protein digestibility corrected amino acid scores (Hoffman & Falvo, 2004; Reeds, Schaafsma, Tome, & Young, 2000; Schaafsma, 2000). Therefore, selecting the right combination of crops that will meet protein requirements is essential. In general, legumes are a major source of protein in parts of South Asia and Sub-Saharan Africa. Among them, pigeonpea occupies an important place, especially in India where it is a major source of protein for vegetarians.

India is the largest producer and consumer of pulses (Gowda, Srinivasan, Gaur, & Saxena, 2013); it accounts for 90% of the world's pigeonpea production (Salunkhe, Chavan, Kadam, & Reddy, 1986). Pigeonpea is also produced in Myanmar, Tanzania, Mozambique, Malawi, and Sudan, the top five exporters of the crop to India (Ahlawat, Sharma, & Singh, 2016; Saxena et al., 2010). Pigeonpea contains 20%–22% protein and has an amino acid profile that compares closely with that of soybean, with the exception of methionine (0.87 vs. 1.55 g/100 g) and cysteine (0.67 vs. 1.44 g/100 g) content (Faris & Singh, 1990; Longvah, Ananthan, Bhaskarachary, & Venkaiah, 2017; Onweluzo & Nwabugwu, 2009; Singh & Jambunathan, 1982). Histidine is another essential amino acid found in abundance in split pigeonpea (3.16 mg/100 g) compared with white soybean (2.55 mg/100 g).

Similarly, chickpea is another nutritious food commonly eaten around the world. It is also an important component of the diets of those who cannot afford animal-source protein and those who are vegetarian by choice, mostly in central and northern India (Jukanti, Gaur, Gowda, & Chibbar, 2012). Protein content in chickpea varies as the percentage of total dry seed mass differs before dehulling (17%–22%) and after dehulling (25.3%–28.9%). The sulfur-containing amino acids such as methionine and cysteine are less in chickpea too (Zia-Ul-Haq et al., 2007).

The amino acid deficit in pigeonpea and chickpea can be complemented by the use of coarse cereals called millets, which are naturally rich in methionine and cysteine. Pearl millet has an average of 11.6% protein while finger millet has 7.3% protein (Shobana et al., 2013). However, millets contain less lysine amino acid compared to pigeonpea and chickpea. On the other hand, some of the varieties of pearl millet are high in iron (Fe) and regardless of varieties finger millet is high in calcium (Rai et al., 2014; Shobana et al., 2013). However, there is very little scientific evidence to show that a combination of legumes and millets can constitute a source of best quality balanced protein and major micronutrients.

Some animal studies conducted to evaluate the nutritional quality of fermented pigeonpea and fermented millet (FPFM), and sprouted pigeonpea and fermented millet (SPFM) have shown significant growth in 36 weanling male Winster rats (Onweluzo & Nwabugwu, 2009). In another study, pearl millet protein digestibility tested in rats was reported up to 97% (Singh, Singh, Eggum, Kumar, & Andrews, 1987); digestibility rate and it was found to be almost the same in low or high protein genotypes.

So, what is the right combination of millets and legumes that can complement each other in terms of high-quality protein and micronutrients? Protein digestibility is mainly related to the level of release and availability of amino acids for absorption in the small intestine and it determines protein quality. Protein digestibility can be evaluated using both in vitro and in vivo methods. The in vivo method is time-consuming and expensive compared to in vitro studies that are equally reliable and used successfully (Ali, Tinay, Ahmed, & Babiker, 2009). To our knowledge, there is no study yet on protein digestibility of finger millets and pearl millet in combination with pigeonpea or chickpea, as the focus has largely been on milk products and soybean or any other crops independently. Considering the importance of these crops especially in developing countries of Africa and Asia where undernutrition and dietary micronutrient deficiencies such as iron and zinc, it is important to reveal the complete nutrient information of these crops not only independently but also in combination to promote its consumption and also to ensure the high dietary nutritional value. Therefore, the aim of the study was (a) to determine amino acid composition of high nutrient varieties of commonly used pigeonpea, chickpea, finger millet, and pearl millet and their complementary potential of each other to give protein and micronutrients (b) to determine protein quality of various legume–cereal combinations by in vitro protein digestibility (IVPD), and (c) to determine effect of cooking on quality of protein and quantity required to meet the Daily Value (DV).

2 | MATERIALS AND METHODS

2.1 | Varieties used

Selected samples of commonly produced varieties of pigeonpea (Asha and Maruti), chickpea (JG 11 and JAKI), finger millet (GPU 28 and VR 847), and pearl millet (Proagro 9444 and Dhanashakti) were obtained from the field and processed for testing, as described below.

2.2 | Protein and micronutrient profiling

One hundred grams of all eight samples were subjected to amino acid profiling using high-performance liquid chromatography (HPLC) method (SGS, India). Apart from the individual varieties, a combination of millets and legumes was prepared in a ratio of 25:75, 50:50, and 75:25 for total protein assay and micronutrient assays, specifically iron (Fe), zinc (Zn), and calcium (Ca). Total protein in grain samples was estimated using Sulfuric acid-selenium digestion method (Sahrawat, Ravi Kumar, & Murthy, 2002). (total N estimated in Skalar Autoanalyzer and protein percentage calculated as $N\% \times 6.25$ conversion factor).

Grain micronutrients such as Fe, Zn, and Ca were analyzed following the method described by Wheal, Fowles, and Palmer (2011). Briefly, grain samples were oven-dried overnight at 85°C prior to digestion, ground enough to pass through a 1 mm stainless steel sieve using a Christie and Norris hammer mill, and stored in screw-top polycarbonate vials. Grain samples were digested with a diacid (nitric and perchloric acid) mixture. After digestion, the volume of the digest was made to 25 ml using distilled water, and the content was agitated for 1 min by a vortex mixer. These digests were used for micronutrient determination using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

2.3 | In vitro protein digestibility using pepsin

Protein digestibility was conducted on three types of samples. Each sample of millets and legumes selected for this study was milled into flour, and then, 10 grams of each sample was subjected to in vitro protein digestibility in replicates as described in the next para. The milled flours were mixed in four different combinations, which included finger millet with pigeonpea, finger millet with chickpea, pearl millet with pigeonpea, and pearl millet with chickpea. All these combinations were prepared in three different ratios, 25:75, 50:50, and 75:25. The uncooked flour samples were then subjected to protein digestibility. The millet with legume combination mixtures in three different ratios was cooked in replicates into porridge and then subjected to protein digestibility.

In vitro protein digestibility by pepsin was conducted using the method described by Maliwal (1983) with the modifications indicated by Monjula and John (1991). A known weight of the sample containing 16 mg nitrogen was taken in triplicate and hydrolyzed with 1 mg of pepsin in 15 ml of 0.1 M HCL at 37°C for 2 hr (Ali et al., 2009). The reaction was terminated by the addition of 15 ml of 10% w/v trichloroacetic acid (TCA). The mixture was filtered quantitatively through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro-Kjeldahl method. Digestibility was calculated using the following formula:

Protein digestibility (%) = N in supernatant − N in blank/N in sample × 100.

2.4 | Simple cooking methods to determine edible quantities of millets on a daily basis

When it comes to consumption, there is very little knowledge on the link between edible quantity of millets and legumes and their nutritional value. To establish this link, a small-scale experiment was conducted to determine how much of pearl millet or finger millet can be consumed by a normal healthy adult and whether the consumable amount can provide adequate Daily Value (DV) of nutrients based on WHO (2002) specifications. In order to establish this, 100 g of pearl millet was cooked in two different ways to determine the quantity of the end product in order to determine the minimum amount that can be eaten when cooked.

1. In the first method, 100 g pearl millet was soaked for 2 hr and then pressure cooked for 15 min by adding two volumes of water. After cooking, it was weighed to determine the cooked quality of pearl millet.

TABLE 1 Major nutrition profile and Protein digestibility of legumes and millets

Crop	Line/variety	Parameters tested: Iron (Fe) (mg/100 g)	Zinc (Zn) (mg/100 g)	Calcium (Ca) (mg/100 g)	Total protein (%)	Average protein digestibility ± *SD*
Pigeonpea	Maruthi	4.08	3.55	191.93	17.00	70.22 ± 0.04
	Asha	3.48	2.63	117.07	16.72	80.36 ± 5.06
Chickpea	JG11	5.50	3.24	205.50	22.31	80.80 ± 0.88
	Jaki	5.40	3.42	162.92	14.47	88.98 ± 2.42
Pearl millet	Dhanshakthi	8.48	5.54	30.04	10.53	95.36 ± 6.26
	Proagro9444	4.73	4.32	22.44	9.06	57.35 ± 16.41
Finger millet	VR847	2.86	2.00	359.79	6.34	47.29 ± 5.13
	GPU28	2.64	2.02	450.33	6.31	47.70 ± 3.98

2. In the second method, 100 g of pearl millet was milled to give 100 g of pearl millet flour, to which ¼ teaspoon of salt and ½ teaspoon of butter were added and mixed with warm water (54 ml) until it became a smooth softball that was then flattened and toasted on a pan.

2.5 | Data analysis

The differences in protein concentration and pepsin digestibility among samples were analyzed using two-way ANOVA. A paired *t* test was performed between varieties within crops to know the significant variation in proteins and amino acids profiles. All descriptive statistics were performed using SATA version.

3 | RESULTS

3.1 | Nutritional variability

There was significant variation in protein and mineral content in all the tested varieties of all the crops (Table 1). The protein level varied from 6.31% in finger millet to 22.31% in chickpea. The average protein content was 16.86% in pigeonpea, 18.39% in chickpea, 9.79% in pearl millet, and 6.32% in finger millet. Variability for Ca content was low—from 22.4 mg/100 g in pearl millet to a high 450.32 mg/100 g in finger millet. Higher Fe and Zn content were recorded in pearl millet (Fe: 8.47 mg/100 g, Zn: 5.54 mg/100 g) and the lowest in finger millet (Fe: 2.64 mg/100 g, Zn: 2.00 mg/100 g).

3.2 | Nutritional pattern in crop mixture/blends

The Dhanashakti variety of pearl millet alone had 8.48 mg/100 g of Fe, and Maruthi variety of pigeonpea had Fe of 4.08 mg/100 g (Table 1). The combination of Dhanashakti and Maruthi in ratios of 75:25, 50:50, and 25:75 showed 7.46, 6.28, and 5.17 mg of Fe/100 g, respectively (Table 2). Similarly, JG 11 variety of chickpea alone had Fe content of 5.5 mg/100 g. The combination of Dhanashakti and JG 11 in the ratios of 75:25, 50:50, and 25:75 showed Fe content of 7.58, 7.07, and 6.45 mg/100 g, respectively (Table 2).

On the other hand, finger millet variety GPU 28 had Ca content of 450 mg/100 g, Maruthi had Ca content of 191 mg/100 g, and JG 11 had Ca content of 205 mg/100 g (Table 1). When finger millet was mixed with pigeonpea (Maruthi) in the ratios of 75:25, 50:50, and 25:75, Ca content was 386.34, 322.8, and 257.18 mg/100 g, respectively (Table 2). Similarly, finger millet with chickpea in combinations of 75:25, 50:50, and 25:75 gave 404.57, 348.61, and 257.67 mg/100 g of Ca, respectively.

The protein content in Maruthi variety of pigeonpea and JG 11 variety of chickpea was 17.00% and 22.31%, respectively. When pearl millet Dhanashakti (10.53% of protein) was added to pigeonpea Maruthi in the ratios of 75:25, 50:50, and 25:75, the protein content of the mixture was reduced and ranged between 12.5% and 15.42% (Table 2). This can provide up to 28% DV for adolescents and 25% DV for adults of 60 kg body weight based on the Recommended Dietary Allowance (RDA) as per ICMR (ICMR, 2010). Similarly, adding Dhanashakti to chickpea variety JG 11 in the ratios of 75:25, 50:50, and 25:75 provided protein in the range of 13.69% and 16.30%, which is 29% DV for adolescents and 27% DV for adults of 60 kg body weight. The overall protein content in the mixture was less than the protein content in pigeonpea alone or chickpea alone (Tables 1 and 2), but it does provide complete protein.

Similarly, GPU 28 variety of finger millet alone had 6.31% of protein. But, when mixed with pigeonpea or chickpea in three different ratios, protein content ranged between 9.58% and 14.82% and 9.59% and 15%, respectively (Tables 1 and 2).

TABLE 2 Protein and major micronutrient content in different combinations of millets and legumes

PM (% w/w)	PP (% w/w)	Protein (%)	Fe (mg/100 g)	Zn (mg/100 g)	Ca (mg/100 g)	Average PD of uncooked mixture ± *SD*	Average PD of cooked mixture ± *SD*	Increase in digestibility (%)
75	25	12.45	7.47	4.96	69.91	35.55 ± 1.24	66.30 ± 0.08	86.50
50	50	12.98	6.28	4.44	96.41	39.61 ± 0.53	61.39 ± 1.26	54.97
25	75	15.42	5.17	3.90	128.59	41.62 ± 0.02	48.46 ± 2.13	16.43
FM (% w/w)	**PP (% w/w)**	**Protein (%)**	**Fe (mg/100 g)**	**Zn (mg/100 g)**	**Ca (mg/100 g)**	**Average PD of uncooked mixture (*SD*)**	**Average PD of cooked mixture (*SD*)**	**Increase in digestibility (%)**
75	25	9.58	3.08	2.51	386.34	26.40 ± 0.83	43.60 ± 0.05	65.16
50	50	12.84	3.45	2.91	322.80	42.47 ± 0.07	66.68 ± 1.02	56.98
25	75	14.82	3.68	3.11	257.18	52.76 ± 1.28	70.30 ± 2.00	33.25
PM (% w/w)	**CP (% w/w)**	**Protein (%)**	**Fe (mg/100 g)**	**Zn (mg/100 g)**	**Ca (mg/100 g)**	**Average PD of uncooked mixture (*SD*)**	**Average PD of cooked mixture (*SD*)**	**Increase in digestibility (%)**
75	25	13.69	7.58	4.66	87.32	53.35 ± 2.18	68.77 ± 2.20	28.90
50	50	14.59	7.08	4.23	111.20	56.04 ± 1.03	70.10 ± 3.04	25.09
25	75	16.30	6.46	3.84	151.88	70.66 ± 0.05	78.28 ± 1.28	10.78
FM (% w/w)	**CP (% w/w)**	**Protein (%)**	**Fe (mg/100 g)**	**Zn (mg/100 g)**	**Ca (mg/100 g)**	**Average PD of uncooked mixture (*SD*)**	**Average PD of cooked mixture (*SD*)**	**Increase in digestibility (%)**
75	25	9.59	3.54	2.55	404.57	60.38 ± 3.13	66.20 ± 0.06	9.64
50	50	12.71	3.95	2.60	348.62	64.72 ± 0.04	67.74 ± 2.19	4.67
25	75	15.00	4.67	2.91	259.67	71.76 ± 0.23	77.00 ± 1.01	7.30

Note: 1: varieties used; Pigeonpea—Maruthi; Chickpea—JG11; Pearl millet—Dhanashakti; Finger millet—GPU 28
Abbreviations: CP, chickpea; FM, finger millet; PD, protein digestibility; PM, pearl millet; PP, pigeonpea; SD, standard deviation

TABLE 3 Amino acid[a] profile (g/100 g of protein) of the millets and legumes

Crop	Line/variety	Aminoacid profile (Mean of R1 and R2)[b] His	Lys	Leu	Isoleu	Phyl	Val	Threo	Tryp	Met	Met + Cys
Pigeonpea	Maruthi	3.98	7.45	7.10	4.22	9.03	5.13	3.82	0.46	0.39	0.97
	Asha	4.28	7.56	7.50	4.43	9.61	5.17	0.76	0.41	0.41	0.93
Chickpea	JG11	3.00	7.69	8.02	5.02	6.08	5.08	3.91	0.94	0.37	1.03
	Jaki	2.94	7.90	7.83	4.95	5.75	5.09	4.15	0.96	0.33	0.84
Pearl millet	Dhanshakthi	2.89	4.02	9.99	4.59	5.30	6.20	3.97	0.66	1.04	1.51
	Proagro9444	2.15	2.81	7.56	3.42	4.14	4.53	2.87	1.00	0.83	1.66
Finger millet	VR847	2.93	4.57	9.14	4.39	5.30	6.45	4.39	0.55	1.83	2.47
	GPU28	2.85	4.00	9.77	4.62	5.31	6.69	4.15	0.70	1.77	2.23
	Coefficient of Variance	11.90	9.15	15.47	13.68	11.72	13.86	14.28	13.20	15.42	4.13
	SE	±0.26	±0.37	±0.91	±0.43	±0.52	±0.54	±0.35	±0.07	±0.87	±1.45
	F statistics	0.92**	8.74**	2.45[NS]	0.50[NS]	7.55**	1.24[NS]	2.86**	0.11**	0.78**	0.79**

[a]Amino acids: His—Histidine, Lys—Lysine, Leu—Leucine, Isoleu—Isoleucine, Phyl—Phenylalanine, Val—Valine, Threo—Threonine, Tryp—Tryptophan, Met—Methionine, Met + Cys—Methionine + Cysteine.
[b]R1—Replicate 1, R2—Replicate 2; NS = nonsignificant.
**$p < .01$

3.3 | Protein quality of millets and legumes

The in vitro protein digestibility test showed that pearl millet variety Dhanashakti had high pepsin digestibility (95.36%). Pigeonpea and chickpea also had high protein digestibility. However, the GPU 28 variety of finger millet had protein digestibility of >45% and <50% (Table 1).

When Maruthi (pigeonpea) was mixed with GPU 28 (finger millet) in the ratio of 25:75, protein digestibility was 26.40%, which increased when the proportion of pigeonpea increased to 75%. However, cooking the mixture as porridge further increased the digestibility of the mixture between 17% and 24%, and this increase seems to be associated with the increase in the ratio of pigeonpea. Similarly, when Maruthi was mixed with Dhanashakti (pearl millet) in the ratio of 25:75, pepsin digestibility was 35.55%, which increased when the proportion of pigeonpea increased to 75%. However, cooking the mixture as porridge further increased the digestibility of the mixture up to 86.5%, and this increase seems to be associated with an increase in Dhanashakti in the mixture (Table 2).

On the other hand, when J 11 (chickpea) was mixed with GPU 28 (finger millet) in the ratio of 25:75, pepsin digestibility was 60.38%, which kept increasing with increased

TABLE 4 Percentage Daily Value (%DV) of essential amino acids that an adult weighing 60 kg and an 18-month-old child weighing 10.8 kg can obtain from 100 g of millets and legumes on dry weight basis

Line/ Variety	Histidine % DV Adult	Histidine % DV Child	Lysine % DV Adult	Lysine % DV Child	Leucine % DV Adult	Leucine % DV Child	Isoleucine % DV Adult	Isoleucine % DV Child	Phenylalanine % DV Adult	Phenylalanine % DV Child
Maruthi	109.17	409.38	68.06	255.21	50.00	201.72	57.92	239.66	99.33	346.51
Asha	111.67	418.75	65.83	246.88	50.21	202.59	57.92	239.66	100.33	350.00
Proagro	32.50	121.88	14.17	53.13	29.27	118.10	25.83	106.90	25.00	87.21
Dhanshakti	50.83	190.63	23.61	88.54	45.09	181.90	40.42	167.24	37.33	130.23
JG11	87.50	328.13	75.00	281.25	60.04	242.24	73.33	303.45	71.00	247.67
JAKI	73.33	275.00	65.56	245.83	50.00	201.72	61.67	255.17	57.33	200.00
VR 847	26.67	100.00	13.89	52.08	21.37	86.21	20.00	82.76	19.33	67.44
GPU 28	30.83	115.63	14.44	54.17	27.14	109.48	25.00	103.45	23.00	80.23
T statistics	−5.27**		−4.28**		−8.52**		−6.37**		−4.59**	

**$p < .001$; %

proportion of chickpea. However, cooking the mixture as porridge increased the digestibility of the mixture between 4% and 9%. Similarly, when J 11 was mixed with Dhanashakti (pearl millet) in the ratio of 25:75, pepsin digestibility was 53.35%, which kept increasing with an increase in the proportion of chickpea. However, cooking the mixture as porridge further increased the digestibility of the mixture between 10% and 29%, and this increase seems to be associated with Dhanashakti.

3.4 | Daily Value (DV) of amino acids from millets and legumes

The amino acid profiles of two pigeonpea varieties and two chickpea varieties (Table 3) show they contain high amounts of all the essential amino acids, except methionine. Similarly, finger millet and pearl millet amino acid profiles showed 50% more methionine levels compared to pigeonpea and chickpea (Table 3). On the other hand, lysine amino acid content was higher in pigeonpea and chickpea (7.45–7.90 g/100 g) varieties compared with 2.81 g/100 g to 4.57 g/100 g in millets (Table 4). The amino acid profile of pigeonpea and chickpea show that they can contribute 50%–100% DV (for a 60 kg adult) of various essential amino acids except methionine (Table 4) and can meet more than 100% DV of essential amino acids for growing children of 10.8 kg body weight, with the exception of methionine (Table 4). When compared to legumes, pearl millet and finger millet contribute 16%–20% of DV of methionine for adults and 30%–50% of DV of methionine for growing children (Table 4).

The cooking method of pearl millet showed that when 100 g of pearl millet was soaked and pressure cooked, it gave 200 g of boiled pearl millet. On the other hand, from 100 g of pearl millet flour, 2 Indian rotis (unleavened flatbread) were made (each weighing 78 g and of 16 cm diameters before toasting). After toasting, the weight of each roti was 58 g.

4 | DISCUSSION

Each crop has advantages in terms of nutritional qualities besides lacks in a few essential minerals and amino acids. The study tested various combinations of millets and legumes to ascertain whether these combinations complement each other in terms of nutrients. Legumes had the highest proteins in total percentage and moderate Ca levels, while pearl millet recorded higher Fe, Zn, Ca, and moderate proteins. The highest Fe and Zn reported was in pearl millet, largely due to an Fe/Zn-enriched (biofortified) variety called Dhanashakti. The variability for proteins and minerals found in this study was concomitant with earlier studies in pigeonpea, chickpea (Saxena et al., 2010), pearl millet (Shobana et al., 2013), and finger millet (Shobana et al., 2013).

The protein digestibility of millets and legumes shows that the protein quality of pigeonpea and chickpea varieties selected was high. Similarly, Dhanashakti variety of pearl millet was not only high in Fe, and its protein quality was also very high (>90%) compared to Proagro. This goes to show that protein quality may vary with variety; hence, it is very important to have this information while formulating recipes. Similar studies conducted in different varieties of pigeonpea show variations in pepsin digestibility not only based on the variety but also on the different processing methods (Duhan, Khetarpaul, & Bishnoi, 2000). The cooking process increases protein digestibility in all

Valine		Threonine		Tryptophan		Methionine		Methionine + Cysteine	
% DV		% DV		% DV		% DV		%DV	
Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child
54.17	222.37	70.00	262.50	31.25	108.70	10.83	28.26	107.93	422.35
51.92	213.16	13.33	50.00	27.08	94.20	10.83	28.26	102.87	402.55
26.28	107.89	28.89	108.33	37.50	130.43	12.50	32.61	184.48	721.86
41.99	172.37	46.67	175.00	29.17	101.45	18.33	47.83	168.30	658.57
57.05	234.21	76.11	285.42	68.75	239.13	10.83	28.26	114.40	447.64
48.72	200.00	68.89	258.33	58.33	202.90	8.33	21.74	92.84	363.27
22.44	92.11	26.67	100.00	12.50	43.48	16.67	43.48	272.26	1,073.18
27.88	114.47	30.00	112.50	18.75	65.22	19.17	50.00	247.89	969.99
−8.43**		−5.34**		−5.23**		−9.40**		−6.53**	

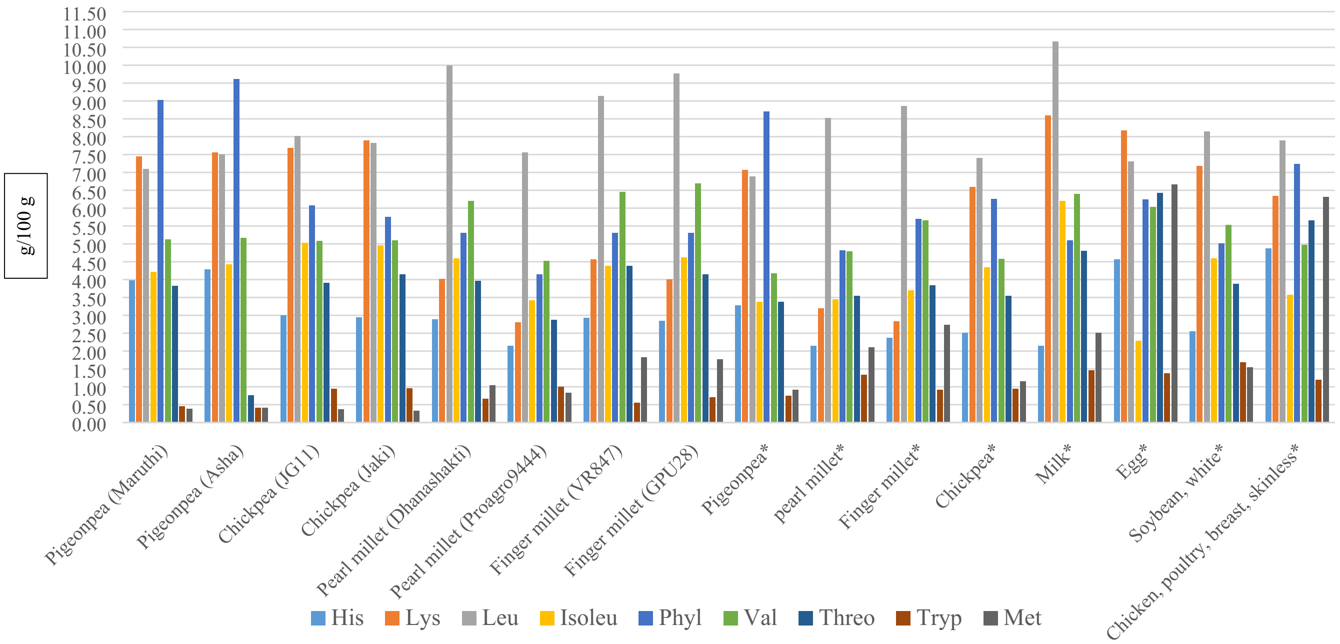

FIGURE 1 Amino acid profile of tested legumes and millets in comparison with available reference data (Longvah, Ananthan, Bhaskarachary, & Venkaiah, 2017).

the combinations. However, pigeonpea and pearl millet have high protein quality and increased digestibility after cooking. A similar study conducted earlier reported similar and comparable protein digestibility in pearl millet (Ejeta, Hassen, & Mertz, 1987; Gulati et al., 2018).

The amino acid profiles indicate the presence of adequate balanced proteins in some of these cultivars which are actually bred for grain yield and not for their nutritional qualities. If crop-specific native nutritional properties brought or conserved through the crop domestication process, then there is a possibility to breed higher nutritional quality along with grain yields. For instance, finger millet is a good source of Ca, certain varieties of pearl millet are a good source of Fe and Zn while pigeonpea and chickpea are the best source of proteins and have moderate amount of Fe and Ca/100 g of grain on dry weight basis. All these crops are grown in several countries of Africa and Asia; however, their full nutritional values either individually or in combination are not well recognized for food preparation yet.

The amino acid profile of pigeonpea and millets is comparable to the reference data that are already available from other studies (Faris & Singh, 1990; Longvah et al., 2017). Some of the currently tested varieties of finger millet and pearl millet are equal or high in amino acids compared to the varieties tested in the Indian Food Composition Table (Longvah et al., 2017). Both the varieties of finger millet studied have comparable amounts of sulfur-containing amino acids (methionine + cysteine) compared to soyabean (2.47 and 2.23 vs. 2.99 g/100 g). Animal-source foods are extremely high in these amino acids (Figure 1). However, finger millet or pearl millet contains 100% or more of DV of sulfur-containing amino acids (which includes methionine and cysteine). Similarly, all the varieties of millets and legumes tested had slightly higher essential amino acids except sulfur-containing amino acids compared to the reference samples. The protein content of variety JG 11 was 22.31 g/100 g, which is higher than that in the reference chickpea, egg, and milk and equal to that in chicken (Faris & Singh, 1990; Longvah et al., 2017). Similarly, pigeonpea protein content is higher than the reference egg and milk (Figure 1). This shows that some of these tested varieties of pearl millet (Dhanashakti) are superior in nutrient content compared to other varieties tested so far. Similarly, 100 g of finger millet is superior in meeting 100% DV of methionine; so, when these varieties are mixed with legumes in adequate quantities, they could form high-quality nutritious food. For example, pearl millet (Dhanashakti) when mixed with chickpea in the ratio of 3:1 (150 g pearl millet with 50 g of chickpea), can more than meet 100% DV of all the amino acids including sulfur-containing amino acids (Table 4). This combination also brings plenty of micronutrients; it can especially provide the required amount of Fe considering only 7.5% of Fe is bioavailable from plant-based food (Table 1). These can be suggested for wider utilization in food preparation.

Our results show that combining Dhanashakti variety of pearl millet with Maruthi variety of pigeonpea or JG 11 variety of chickpea increased the percentage of Fe with an increase in the amount of pearl millet in the mixture (Table 5). At the same time, the percentage of protein in the mixture decreased when the ratio of pigeonpea or chickpea to millets decreased (Table 5). Similarly, combining finger millet with pigeonpea or chickpea increased the Ca level up to 52.91%

TABLE 5 Increase in iron (Fe) and calcium (Ca) percentage and decrease in protein percentage when pearl millet or finger millet added to pigeonpea or chickpea

Fe in PP (mg/100 g)	Fe in PP: PM mixture (mg/100 g)	Increase in Fe in the mixture (%)
4.08	5.17 (75:25)*	26.72
4.08	6.28 (50:50)	53.92
4.08	7.46 (25:75)	82.84
Fe in CP (mg/100 g)	**Fe in CP:PM mixture (mg/100 g)**	**Increase in Fe in the mixture (%)**
5.5	6.45 (75:25)	17.27
5.5	7.07 (50:50)	28.55
5.5	7.58 (25:75)	37.82
Ca in PP (mg/100 g)	**Ca in PP:FM mixture (mg/100 g)**	**Increase in Ca in the mixture (%)**
191.93	257.18 (75:25)	25.37
191.93	322.80 (50:50)	40.54
191.93	386.34 (25:75)	52.91
Ca in CP (mg/100 g)	**Ca in CP:FM mixture (mg/100 g)**	**Increase in Ca in the mixture (%)**
205.50	257.67 (75:25)	20.25
205.50	348.62 (50:50)	41.05
205.50	404.57 (25:75)	49.21
Protein in PP (mg/100 g)	**Protein in PP:PM mixture (mg/100 g)**	**Decrease in protein in the mixture (%)**
17	15.42 (75:25)*	9.29
17	12.98 (50:50)	23.65
17	12.45 (25:75)	26.47
Protein in CP (mg/100 g)	**Protein in CP:PM mixture (mg/100 g)**	**Decrease in protein in the mixture (%)**
22.31	16.30 (75:25)	26.94
22.31	14.59 (50:50)	34.60
22.31	13.69 (25:75)	38.64
Protein in PP (mg/100 g)	**Protein in PP:FM mixture (mg/100 g)**	**Decrease in protein in the mixture (%)**
17.00	14.82 (75:25)	14.71
17.00	12.84 (50:50)	32.40
17.00	9.58 (25:75)	77.45
Protein in CP (mg/100 g)	**Protein in CP:FM mixture (mg/100 g)**	**Decrease in protein in the mixture (%)**
22.31	15.00 (75:25)	48.73
22.31	12.71 (50:50)	75.53
22.31	9.59 (25:75)	132.63

Note: Abbreviations and varieties tested: PP = pigeonpea—Maruthi; CP = chickpea—JG11; PM = pearl millet—Dhanashakti; FM = finger millet—GPU 28.

*Figures in parentheses are the ratio of mixture.

and 49.21%, respectively in the formulation as the amount of finger millet increased from 25% to 75%. At the same time, the level of protein decreased by up to 77.45% when the ratio of finger millet increased in pigeonpea, and it decreased by up to 132.6% when the finger millet ratio increased in the chickpea mixture (Tables 5). Despite the decrease in protein content, they can still provide the required amino acids and micronutrients if they are consumed in the right proportion and in adequate quantities.

Locally available home food processing methods alter the quantity of the end product. For example, when pearl millet is soaked for 2 hr and pressure cooked for 15 min, the quantity of the end product increases on an average by 100% (100 g of pearl millet becomes around 200 g after boiling). This is because soaking and then boiling softens the pearl millet grain and it absorbs some water and bulges. Therefore, in this form, the dry weight to cooked weight doubles and cannot normally be consumed by any adult (200–300 g of pearl millet in boiled form becomes about 400–600 g of cooked grain). However, if the same pearl millet is made into raw flour and cooked into a roti, then the quantity of the end product does not change much from the quantity of raw pearl millet used. This is because of the low water absorption capacity of raw flour compared to boiled or pressure cooked millet. A similar study was conducted on barnyard millet and foxtail millet that showed dry flour has low water absorption capacity (Nazni & Shobana Devi, 2016). A normal adult can easily consume 200 g of pearl millet (on dry weight basis) in the form of roti. For example, 200 g of pearl millet flour can make four medium-sized rotis of 60 g each and can be consumed easily by any normal adolescent and adult together with vegetables and legumes. So, if an adult can consume 200 g of pearl millet, then she/he can obtain up to 48% of DV of methionine. Children of 10.65 kg weight can obtain 100% DV of methionine and also required Fe.

A previous study to develop complementary food in Nigeria using flour blends of sorghum, pigeonpea, and soybean in ratios of 71.4:14.4: and 14.3 showed high total protein content (22.63%) compared to other proportions that were tested (Adeola, Shittu, Onabanjo, Oladunmoye, & Abass, 2017). In another study, 36 weanling male Winster rats fed on fermented pigeonpea and fermented millet (FPFM) and sprouted pigeonpea and fermented millet (SPFM) composite showed highest ($p < .05$) weight gain (113.51 g and 123.42 g), protein efficiency ratio (2.15 and 2.02), biological value (70.7 and 76.2), Net Protein Utilization (70.13 and 74.57) and promoted growth better than other formulated diets (Onweluzo & Nwabugwu, 2009). This shows that nutritious weaning diets can be formulated by complementing unexploited legumes and cereals like pigeonpea and millets as a potential source of protein. Moreover, they can also complement each other for dietary micronutrients such as Fe, Ca, and Zn.

In the current study, various ratios of legumes and millets were combined in such a way the mixture would provide both protein and micronutrients. Pearl millet variety Dhanashakti was found to contain 8.48 mg/100 g of iron; its amino acid profile shows that consuming 150 g of pearl millet can meet 100% DV of methionine in weaning children. Moreover, consuming pigeonpea or chickpea along with pearl millet can provide a complete protein with all essential amino acids (Table 4).

Bioavailability of micronutrients and amino acids was not studied and is a limitation of this study, but there is evidence to show that the consumption of 159 g of pearl millet varieties gives approximately 7.5% of fractional Fe bioavailability which can meet 72% of median daily Fe requirement of women above 18 years of age (Cercamondi et al., 2013). Another study conducted by Finkelstein et al. (2015) gave us strong and promising evidence indicating that Fe-rich pearl millet varieties (8.6 mg/100 g) can help in treating Fe deficiency and in reducing iron deficiency anemia fairly quickly compared to low-Fe pearl millet (2.1 mg/100 g). Both the efficacy studies used high- and low-Fe varieties of pearl millet and claim almost no differences in nutrients' bioavailability between both types, implying the presence of genetic variability for nutritional traits among varieties while genetic variability for bioavailability is negligible. Considering the 7.5% of bioavailability that was obtained in these studies, using high-Fe varieties like Dhanashakti (8.48 mg) when consumed adequately (200–300 g/day) could help in reducing iron deficiency anemia. This was proven in a study conducted by Finkelstein et al. (2015). Children fed the normal quantity of 200–300 g/day met with Fe requirement but it also led to a median change in Serum Ferritin (SF) levels in the first 4 months of the study. SF levels were significantly high ($p < .05$) with 5.7 mg/L (34.9% increase) in children who consumed high-Fe pearl millet against 1.2 mg/L (7.3% increase) in those who had consumed low-Fe pearl millet (Finkelstein et al., 2015).

5 | CONCLUSIONS

The current study provides preliminary evidence that a combination of millets with a legume naturally provides high-quality complete protein with high digestibility and Fe or Ca when consumed in an adequate quantity in the ratio of 3 portions of millet and one portion of legume. This is not possible with polished rice, refined wheat flour or maize even if it is combined with legumes, as these cereals are inherently deficient in amino acids and micronutrients compared to millets. This study provides a rationale to bring back millet as a staple food considering the high protein malnutrition and iron deficiency anemia in different parts of the developing world. This study has also identified the limitations and opportunities for further research that can support further discovery and scaling up. All the millets and grain legumes from different geographical regions should be tested to confirm the optimal combinations that will maximize quality protein. Bioavailability studies are available for nutrients in pearl millet and protein in legumes; however, such study in their combination food products may be required. The right combination of legumes and millets has prospects of enhancing the nutritional value of foods and contributing to a balanced dietary system to combat protein and micronutrient malnutrition, especially among children and women.

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