# Proximate and Mineral Nutrition of Common Bean Genotypes as Influenced by Harvesting Time

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# Abstract

Common bean is the most consumed legume by humans and hence significant for global food security and nutritive value mainly iron (Fe) and zinc (Zn). The objective of the study was to evaluate the effect of harvesting time and genotypes on proximate and mineral composition of common bean. Two field experiments were carried out in Sebele ( $24^{\circ}33'S$ ,  $25^{\circ}54'E$ , 994 m above sea level) horticultural fields during summer 2018/2019. Treatments included two common bean genotypes DAB 564 (white) or 520 (red) and three harvesting stages as the early, mid and late season harvest. In both seasons, genotype and harvesting time significantly (P < 0.05) influenced proximate, macro and micro mineral composition of common bean seeds. Generally genotypes studied had a sufficient amount of proximate and mineral content with crude protein, iron (Fe) and zinc (Zn) content as the most abundant minerals at a range between 20-22%, 33-101 mg g<sup>-1</sup> and 41-45 mg g<sup>-1</sup>, respectively across harvesting times. Genotype DAB 564 (white coloured) significantly (P < 0.05) produced seeds with the highest concentration of proximate and mineral contents especially zinc (45 mg g<sup>-1</sup>) and iron (70 mg g<sup>-1</sup>) content irrespective of season. Harvesting common bean seed early or mid-stage produced high seed concentration of proximate, micro and macro mineral content across genotype or season.

Keywords: common bean, genotypes, harvesting time, zinc, iron, crude protein

# 1. Introduction

Legumes are staple food in human diets around the world (FAOSTAT, 2010; Ndidi et al., 2014), hence their wide cultivation in most countries with total world production rates of more than 17 million tonnes, led by China, Indonesia, India and Turkey as the largest producers and consumers of beans (FAOSTAT, 2010; Richardson, 2012). Among legumes, *Phaseolus vulgaris* (common bean) is highly adaptive, it is drought, heat tolerant and thrives well in marginal soils. Nutritionally, beans are one of the best non-meat sources of iron providing 23-30% of daily recommended levels from a single serving. Maruapula and Novakofski (2010) has reported iron as the only micronutrient with the intake above the recommended two-thirds of recommended daily allowance (RDA) above making common beans a vital source of iron. Common bean is also rich source of protein, carbohydrates and vitamin B complex with high level of other micro nutrients such as copper, zinc, phosphorus, potassium, magnesium and calcium, high fibre content and a good source of poly unsaturated fatty acids (Kelly & Scot, 1992; Ndengwa et al., 2006).

Despite the nutritional value of legumes including common bean, most African countries including Botswana still experience high incidences of food insecurity, hunger and micronutrient induced malnutrition including zinc and iron deficiencies (Nestel et al., 2006). In Botswana, 29% of children below 5 years have impaired growth and 11% are underweight (UNICEF, 2009). This resulted with 78 million USD of money spent annually for treating vitamin and mineral deficiencies and/or illnesses (UNICEF, 2004, 2009; World Bank, 2009). Also the adult age groups of men and women has poor health conditions which are mostly caused by low macro and micro nutrient intake especially protein, fiber, iron and zinc (Maruapula & Novakofski, 2010). In Africa, South Africa and Botswana has been reported to have iron deficiencies especially on the elderly groups due to consumption of food which do not meet the daily recommended allowances. Maruapula and Novakofski (2010) reported low micronutrient intake of Fe (8 mg day<sup>-1</sup>), Zn (8-11 mg day<sup>-1</sup>) and selenium for men and women in

Botswana which may affect the immune system. The adequate iron intake in most health groups especially the elderly was from beef and sorghum grain with low contribution from fruits and vegetables (Maruapula & Novakofski, 2010). This deficiency incidence is more prominent in infants below 5 and woman and can lead to ailments such as anemia, mental impairment, still births, underweight in infants, blindness and infant mortality. Common bean has been reported to be advantageous for use in the biofortification programs since the baseline grain fe content is high at 55 ppm and variability for the trait is great (Petry et al., 2015).

Besides the food security and health nutritional deficiencies in Botswana, low cultivation rates is also affected by low erratic rainfall, poor soils, high temperatures and droughts. This requires cultivation of hardy crops such as common bean which has an adaptive potential, soil improvement properties and good nutritive value. Studies has reported a wide genetic diversity and variability in growth habit, seed size, shape and colour, maturity and adaptation among common bean genotypes impacting growth, seed yield, quality and nutritional composition (Jones, 1999; Beebe et al., 2000; Tryphone & Nichumbi-Msolla, 2010). The success of seed filling is a great determinant of seed yield, quality and nutrition as influences timing and duration of a crop to reach physiological and harvest maturity. The visual indicator for the time of harvest is usually predicted by the colour of pods from green to yellow or brown (Cerna & Beaver, 1989) and may proceed until the pods are completely dry to shattering (Muasya, 2001). Thus legumes attain maximum seed weight at physiological maturity after which drying begins and seed quality indicators begin to deteriorate depending on the time of harvest (Muasya, 2001; Tekrony & Egli, 1997). Harvest maturity of legumes varies depending on moisture content from 15-20%, 19%, 17-25% and 35-58% for soybean (Copeland & McDonald, 1995), mung bean (Dharmaligam & Basu, 1990), dry bean (Van de Venter et al., 1996; Kelly, 1988) and common bean (Muasya, 2001), respectively. The variation in seed filling time and duration between and within a common bean crop results with concurrent and/or progressive harvesting intervals of common bean seeds hence inconsistency in the seed quality (Muasya, 2001). Simultaneous harvest as practiced by most farmers results in poor seed bulk quality and nutrition (Muasya, 2001; Wortmann, 2006). Therefore this study seeks to assess the seed nutritional concentration among common bean genotype harvested at different stages to establish their optimum time of harvest. The study will determine the nutritive potential of common bean genotypes under Botswana conditions to address the nutritional and health deficient population through evaluating the effect of genotype and harvesting time on proximate and mineral composition.

# 2. Materials and Methods

# 2.1 Location

Field experiments were conducted in Sebele (24°33'S, 25°54'E, 994 m above sea level) horticultural fields in summer 2018 and 2019. The climate in Sebele is semi-arid with an average annual rainfall (30 year mean) of 558 mm (Bekker & de Wit, 1991). Most rain falls in summer, which generally starts in late October and continues to March/April. The mean rainfall ranges between 250-650 mm per annum. The soils are shallow, ferruginous tropical soil, mainly consisting of medium to coarse grain sandy loams with a low water holding capacity.

# 2.2 Experimental Design

The experiment was laid in a randomised complete block design (RCBD) under split-plot arrangement with three replications. The treatments were two common bean genotypes DAB 564 (white coloured) and DAB 520 (red coloured) allocated randomly to main-plots. These genotypes were selected among Andean common bean (Molosiwa et al., 2019). Three harvesting intervals of early (70 days after sowing (DAS)), mid (80 DAS) and late (90 DAS) season harvest were randomly allocated as subplots. The main plot size will be 3 m × 3 m.

# 2.3 Cultural and Management Practices

The land was cleared, ploughed followed by disc harrowing to a fine soil tilth before planting using mould board and disc harrow. Soil was sampled to determine mineral composition prior to planting. The fertilizer application rates for basal dressing were done according to the soil mineral content after soil analysis. Common bean seed was sown directly, 2 seeds placed per seeding point. All necessary management practices including fertilizer application, pests, disease and weed control were undertaken to enhance good growth and development.

# 2.4 Data Collection: Proximate and Mineral Analysis

Common bean seeds will be harvested at different harvesting times during ripening to determine their proximate and mineral content.

# 2.4.1 Dry Matter (DM)

Dry Matter (DM) content was determined according to method 973.18 of the AOAC (2012), through weighing

approximately 1 g of each ground sample into pre-weighed crucibles and placing it in an oven at 60°C for 24 hours. DM was calculated as the difference between initial sample weight and moisture weight and expressed in percentage.

# 2.4.2 Crude Fibre (CF)

Crude Fibre (CF) content was determined by the Weende method (AOAC, 1990), through hydrolysing dried samples with boiling 0.128 M sulphuric acid, followed by boiling in 0.223M potassium hydroxide solution in hot extractor. The residue was washed with preheated distilled water before being transferred to a cold extractor and washed with acetone. The residue and crucibles were oven dried at 105 °C overnight and weighed ( $W_1$ ) before being ignited into a muffle furnace at 450 °C for 8 hrs. The residual ash was first cooled in an oven at 105 °C overnight, then cooled to room temperature in a dessicator and weighed ( $W_2$ ). The percentage of crude fibre was calculated as:

% Crude Fibre = 
$$[(W_1 - W_2)/W_0] \times 100$$
 (1)

# 2.4.3 Crude Protein (CP)

The ground dry sample was digested in a BD block at 330 °C for 7 hrs. After digestion nitrogen (N) was determined through distillation and titration using the micro kjeldahl method number 976.06 (AOAC, 2012) and then converted to crude protein content through multiplying percentage N content by a factor of 6.25 and expressed in %.

# 2.4.4 Ash Content

A finely ground dry sample was weighed into a clean dried crucible which was initially weighed as  $W_1$ . The sample was ignited over a low flame to char the organic matter with lid removed. The crucible was then placed in a muffle furnace at 600 °C for 6 hrs until its completely ashed. Thereafter the sample was transferred into the desiccators, cooled and weighed immediately as  $W_2$ . The percentage ash was calculated as:

% Ash Content = [Weight Loss (
$$W_2$$
) × 100]/Weight of Sample ( $W_1$ ) (2)

# 2.4.5 Mineral Analysis Determination

The samples will be oven-dried at 66 °C to constant weight (72 hours). The dried samples were ground using a sieve of size two and 1.25 g composite sample used digestion. Digestion was done for 3 hours using One Touch Technology oven (MARS). After digestion of samples N, P, K, Na, Ca, mg, Zn and Fe will be determined. Nitrogen (N) was determined through distillation and titration using the micro kjeldahl method (AOAC, 1996). Phosphorus (P) was determined calorimetrically using sodium phenol and ammonium molybdate plus ascorbic acid method (AOAC, 1996). The absorbance was read on the UV Visible Spectrophotometer (Model of spectrophotometer). Calcium (Ca), magnesium (mg), potassium (K), Zinc (Zn) and iron (Fe) was determined by atomic absorption spectrophotometry (Varian SpectrAA 300). Data is expressed as total mineral content in mg/g on dry weight basis.

# 2.5 Statistical Analysis

Analysis of variance was performed on the data collected using general linear model (PROC GLM) procedure of Statistical Analysis System (SAS) 2009 program package, version 9.1. Appropriate regression models were used to examine the response of bean seed genotypes to different harvesting times. Multiple comparisons among means was done using Protected Least Significant Difference (LSD) at p = 0.05. Proc univariate procedure was carried out on residuals to support assumptions of normality made.

# 3. Results and Discussion

Genotype and harvest time had a significant (P < 0.05) effect in the proximate and mineral nutrition of common bean during two seasons of study (Tables 1 and 2). There was significantly (P < 0.05) high dry matter (94%), crude fibre (5%), crude protein (22%) and ash content (5%) from genotype DAB 564 than DAB 520 irrespective of season (Table 1). However, proximate nutrition was high during the first season of cultivation compared to season 2 (Table 1). Crude fibre and protein resulted with 17 or 41% and 6 or 7% genotypic difference, respectively during season 1 or 2 (Table 1), indicating genetic variations with genotype DAB 564 expressing a better nutrition than DAB 520. DAB 564 may genetically have a high photosynthetic activity and partitioning for mineral composition. The seed ash content ranged between 4-5 % indicating that common bean seed genotypes are an important source of minerals.

Basing on nutritional response towards time of harvest, the lowest and highest crude protein content were 19% and 22% from late and early harvest during season 1 or 16% and 23% from late and mid harvest during season

2, respectively (Table 1). However, during the first cultivation early and mid-season harvest did not significantly (P < 0.05) differ (Table 1). In general, mid harvest was consistent in recording the highest seed proximate concentrations even though in some cases there was no significant (P < 0.05) difference between early and mid-harvest towards crude protein and ash content (Table 1).

This indicates a stronger source-sink relationship during early and mid-stages of harvest which means sufficient accumulation and partitioning of photo assimilates hence high seed protein and ash concentration. Muasya (2001) also found a high seed dry matter from early and medium harvested pods than in late harvest irrespective of cultivar. Cocks (1990) also reported an increased seed quality and dry matter content which was related to an increase in a higher seed filling rate at early/mid stages which was attributed to less competition for accumulating insoluble reserves before all seeds are formed.

	Dry Matter (%)		Crude	Crude Fibre (%)		Crude Protein (%)		Ash Content (%)	
	S1	S2	S1	S2	S1	S2	S1	S2	
Genotypes									
DAB 564	93.70a	93.43a	5.42a	5.38a	21.75a	19.90a	4.72a	4.54a	
DAB 520	93.60b	92.33b	3.21b	4.48a	20.13b	18.76b	4.61b	3.97b	
LSD	0.086	0.45	1.23	1.12	1.25	0.80	0.12	0.42	
Significance	*	***	**	NS	*	**	****	**	
Harvesting time									
Early Harvest	93.56b	93.42a	2.19b	6.60a	21.81a	18.89b	4.81a	4.21b	
Mid Harvest	93.71a	93.60a	5.33a	5.27a	21.24ab	22.73a	4.91a	5.29a	
Late Harvest	93.68a	91.61b	4.70a	2.93b	19.67c	16.38c	4.28b	3.26c	
LSD	0.11	0.55	1.51	1.37	1.56	0.98	0.143	0.52	
Significance	*	****	*	***	*	****	*	****	

Table 1. Effect of genotype and harvest time on proximate content of common bean

Note. \*, \*\*, \*\*\*, \*\*\*\* Significance at P = 0.05, 0.01, 0.001, 0.0001, respectively. NS=Not significant.

Means separated using the Least Significant Difference (LSD) at P = 0.05; Means with the same letter(s) are not significantly different.

There was a significant (P > 0.05) effect of genotypes towards all the common bean mineral composition irrespective of season with exception of magnesium during season 1 (Table 2). Comparing genotypes, DAB 564 (white seed) had a high mineral content than DAB 520 (red seed) across seasons (Table 2). In both seasons, iron (Fe) was the most abundant mineral with the lowest and highest values of 53 and 70 mg g<sup>-1</sup> from DAB 520 and DAB 564, respectively (Table 2) making a 24% difference among the two genotypes (Table2). This was similar to the findings by Silva et al. (2012) and McClean et al. (2017). In their findings McClean et al. (2017) observed a high Fe and Zn concentrations in small-white (63/33 g/g) than small red (57/30) genotypes with 9% (Fe) and 10% (Zn) difference between the genotypes. Silva et al., (2012) also reported a high Zn and Fe concentrations in the black market bean genotypes.

From the current study potassium (K), calcium (Ca) and phosphorus (P) resulted with 12, 19 and 53% genotype differences in season 1 while during the second cultivation there was 35%, 19% and 22% difference among genotypes, respectively (Table 2). The significant (P < 0.05) difference among genotypes on micro and macro seed mineral concentration implies that genetic difference of common bean influence its seed nutritional value. The accumulation of mineral elements in the bean seed is influenced by crop growth and development factors which are partially controlled by genes. This has been explained by Conte and Walker (2011) and McClean et al. (2017) that photosynthetic activities are essential for delivery of minerals from the source to the sink tissues (seeds) through the xylem-phloem pathway. This process requires multiple transporters and regulatory pathways such as transcription factors, mRNAs, sugars and hormones specific or shared to a mineral (Lejay et al., 2003; Pottier et al., 2014; Paul et al., 2015). The translocation pathway indicates that genetic components or allelic variation of genes that encode this factors control variability in seed mineral concentrations among genotypes (Ding et al., 2013; Mamidi et al., 2014; Huang et al., 2015). In both seasons the micro and macro mineral elements of calcium (Ca), iron (Fe), potassium (K), phosphorus (P) were significantly (P < 0.05) high in white coloured bean of genotype DAB 564 compared to a red coloured bean of genotype DAB 520 (Table 2). Similar

findings were reported by Bassett (2007) where the white coloured beans had significantly high Ca, Cu, Fe, Mg, Mn, Mo, Ni, P, S compared to coloured beans.

		um (Ca) g g <sup>-1</sup> )	Iron (Fe)	(mg g <sup>-1</sup> )		ium (K) g g <sup>-1</sup> )	1	orus (P) g g <sup>-1</sup> )	U	sium (Mg) g g <sup>-1</sup> )
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Genotypes										
DAB 564	0.38a	0.36a	70.18a	73.12a	1.86a	2.76a	0.16a	0.21a	0.20a	0.23a
DAB 520	0.18b	0.28b	52.83b	67.01b	1.64b	1.80b	0.13b	0.17b	0.19a	0.18b
Significance	*	**	*	*	*	**	*	*	NS	****
LSD	0.19	0.05	15.99	6.10	0.22	0.48	0.03	0.03	0.21	0.01
Harvesting Time										
Early Harvest	0.23a	0.40a	100.58a	73.36b	2.01a	3.05a	0.21a	0.18b	0.19b	0.21b
Mid Harvest	0.23a	0.37a	79.18b	83.85a	1.73b	2.27b	0.16b	0.25a	0.21a	0.23a
Late Harvest	0.21b	0.20b	32.75c	49.99c	1.63b	1.52c	0.08c	0.14b	0.19b	0.19c
Significance	*	***	***	****	*	***	***	***	**	***
LSD	0.02	0.06	19.59	7.49	0.27	0.58	0.04	0.04	0.01	0.02

Table 2. Effect of genotype and harvest time on mineral nutrition of common bean

Means separated using the Least Significant Difference (LSD) at P = 0.05; Means with the same letter(s) are not significantly different; S means Season.

In their findings seed mineral concentration as affected by bean genotypes was genetically controlled by phenotypic difference of colour from a single gene p, all coloured common bean genotypes were found to carry a dominant allele while all white genotypes carried the recessive p allele (Bassett, 2007). This was emphasised by Possobom et al. (2015) and McClean et al. (2002) who studied common bean seed coat and found that colour expression depends on multiple allelles at P locus. This genes (V or vv) regulate flavanols (V) and anthocyanin (vv) biosynthetic pathways (Bassett, 2007; Binger et al., 2000).

There was a significantly (P < 0.05) high seed mineral composition when common bean was harvested early compared to late harvest of season 1 except for magnesium. During season 2, the highest seed mineral concentration were produced during mid than early or late harvest with an exception of potassium (Table 2). However, ca, K and mg bean concentrations experienced a similar response at early or mid-harvest irrespective of season (Table 2). Bean seed iron (Fe) significantly (P > 0.05) ranged between 33 to 101 mg g<sup>-1</sup> or 50-84 mg g<sup>-1</sup> as affected by the time of harvest during the first or second season, with the highest fe value at early or late harvest recording 40-68% nutritional difference among harvesting stages, respectively (Table 2). Early pods benefit from high influx of assimilate when the plant switches from vegetative to reproductive phase (Bewley & Black, 1994). Dornbros Jr. (1995) stated that the earlier the crop is harvested after reaching physiological maturity the better the seed quality. The decrease in mineral seed concentration late during harvest may be explained by a loss in seed quality parameters most of which impact on mineral nutrition. Dornbros Jr. (1995) has reported that after physiological maturity drying begins, triggered by senescence where the seed quality is gradually lost after physiological maturity as the natural seed deterioration progresses. The shorter the period between physiological and harvest maturity, the less seed deterioration rate (Muasya, 2001) and therefore greater seed quality.

Genotype did not significantly (P > 0.05) influence the zinc content of common bean seed however, harvesting time significantly (P < 0.05) influenced zinc concentrations across seasons (Table 3). There was significantly high zinc value when bean seeds were harvested early or mid during season 1 or 2 respectively (Table 3). In both seasons the lowest zinc content was produced late during the season (Table3). Season 1 further revealed a significant interaction (P < 0.05) between genotype and harvest time to influence zinc content of common bean, resulting with a range between 41-45 mg g<sup>-1</sup> (Table 3). The lowest zinc content was 41 mg g<sup>-1</sup> from DAB 564 late in the season, however it did not significantly (P > 0.05) differ with any genotype at any time of harvest except for DAB 564 at early harvest (Table 3). The low zinc content during late harvest of both genotypes may be influenced by senescence interrupting seed filling process of late pods due to the reduced photosynthetic

activities. Wortmann (2006) stated that late harvest during the season could expose seeds to unfavourable conditions resulting in deterioration in seed quality and nutrition, this was also supported by Dornbos Jr. (1995) who reported that after physiological maturity, drying commences and seed quality is gradually lost.

Genotype or Harvest time Effect			<b>Genotype × Harvest time Interaction Effect</b>			
	Saaaan 1	Saagan 2	Construes	Harvesting Time		
	Season 1	Season 2	Genotypes	Season1	Season2	
Genotypes			Early-Harvest			
DAB 564	43.14a	37.73a	DAB 564	45.38a	42.93ab	
DAB 520	42.40a	34.10a	DAB 520	42.08bc	38.77b	
Significance	NS	NS	Mid-Harvest			
LSD	0.23	3.47	DAB 564	42.71bc	45.53a	
Harvesting time			DAB 520	42.97b	44.34ab	
Early-Harvest	43.73a	40.85b	Late-Harvest			
Mid-Harvest	42.84b	44.94a	DAB 564	41.33c	24.75c	
Late-Harvest	41.73c	21.98c	DAB 520	42.14c	19.20c	
Significance	**	****	Significance	**	NS	
LSD	0.20	4.60	LSD	0.99	1.88	

Table 3. Effect of genotype and harvest time on zinc content of common bean

*Note*. \*\*, \*\*\*\* Significance at P = 0.01, 0.0001, respectively. NS = Not significant.

Means separated using the Least Significant Difference (LSD) at P = 0.05; Means with the same letter(s) are not significantly different.

The study indicates that common bean has a sufficient seed mineral content with crude protein, iron and zinc as the abundant proximate and mineral concentrations irrespective of genotype or harvest time. The range between 20-22% crude protein content of bean seed makes it a sufficient protein source for the genotypes studied at any stage of harvest. Pearson (1976) has reported that plant foods should contain more than 12% of its caloric value as a good protein source while the Food and Nutrition Board of Institute of Medicine (2002) reported a daily required intake of crude protein ranging between 9-13 g and 34-56 g for children and adults, respectively. The type of protein contained in beans is reported to be highly digestible for human consumption (Demardi-Blacberry et al., 2004), hence a good source for protein malnutrition. Basing on iron and zinc content, food commodity is regarded a nutrient source for human consumption when 100 g of the product has more than 15% dietary reference intake (DRI) for the desired nutrient. Also DRI for iron and zinc is 14 mg/day and 15 mg/day respectively (Codex Alimentarious Commission/FAO, 1997). From the current study, findings reveal that common bean genotypes DAB 520 and DAB 564 produced fe (41-45 mg  $g^{-1}$ ) and zn (33-103 mg  $g^{-1}$ ) content with 3 or 7 times daily recommended intake at all harvesting stages hence it can be considered as a high Fe and Zn source (Carvalho et al., 2011). Iron and zinc are recommended as essential for proper functioning of the immune system and some human health deficiencies such as anemia (Barclay et al., 1996; Rosado et al., 1992). Also, Rayman (2000), and Tugce et al. (2018) reported a positive correlation (r = 0.541\*\*) between zinc and selenium content, this is an important nutritional element for strengthening immune system and lowering the risk of cancer (Rayman, 2000; Sunde, 1997; Tugce et al., 2018) and HIV/AIDS, a disease known to lower the immune system (Maruapula & Novakofski, 2010). Low incidences of cardiovascular diseases, cholesterol, diabetes and obesity has been related with high bean consumption (Demardi-Blacberry et al., 2004; Schneider, 2002; Suarez-Martinez et al., 2015).

# 4. Conclusion

Proximate and mineral nutrition of common bean is significantly influenced by genotype and harvesting time. Common bean genotypes had the highest proximate and mineral (Ca, Fe, K, P, Zn) seed concentrations during early or mid-harvest compared to a later stage. Genotype DAB 564 is highly nutritive compared to DAB 520. However, the two common bean genotypes studied had sufficient nutrients with crude protein, Fe and Zn as the most abundant ranging up to 22 %, 45 mg g<sup>-1</sup> and 103 mg g<sup>-1</sup>, respectively. The nutritional importance of common bean genotypes DAB 564 and DAB 520 in this study proves that enhancing cultivation of common bean in Botswana will not only promote food security but improve household diets and human health nutrition of

different age and health groups meeting their daily key nutritional requirements. The high iron and zinc levels implies that as a plant based mineral source, common bean genotype DAB 564 has a potential for use in food biofortification to enhance Fe and Zn accumulation especially for infant formulations (such as Tsabana-a meal issued as supplementary diet in health facilities of Botswana).

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