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Chapter 14

Improving Cassava Quality for Poultry Feeding Through Application of Biotechnology

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Abstract

The continuous increase in cost of conventional energy sources caused by inadequate supply and stiff competition between human, animals and various industries for many decades has resulted to the need to source for suitable, readily available and cheap energy sources for poultry production globally. One such alternative is cassava. A native to South America, cassava is now found in abundance in most tropical countries. Due to lack of excellent post-harvest technologies, large quantities of cassava are wasted. An increased use of cassava in poultry feeding will go a long way to reduce this wastage and also reduce the high cost of poultry feed. However, the utilisation of cassava in poultry nutrition has been hindered by its lower nutritional value, especially protein and amino acids, presence of some ANF and dustiness when poultry feed is produced with cassava meal. Traditional processing methods have only succeeded in taking the inclusion level of cassava to 40% in some poultry diets. Researchers and poultry nutritionists have become interested in developing multi-pronged technologies and processing methods to increase cassava utilisation in poultry nutrition to reduce wastage, improve its nutritional value and maximise production. This chapter highlights the application of different technologies and the importance of biotechnology in improving the quality of cassava and increasing its utilisation for poultry feeding.

Keywords: biotechnology, cassava, feeding, nutrition, poultry

1. Introduction

For many decades, global poultry production has been negatively affected by high and increasing cost of feed. This persistent increase in the cost of poultry feed is caused majorly by the escalating cost of conventional energy and protein feed ingredients used to compound

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poultry diets. The continuous increase in the cost of these ingredients could be blamed on the food-feed competition between man, animal and various industries for conventional and available energy and protein sources. This incessant upsurge in the cost of conventional energy and protein source has been and will continue to be a source of dilemma to most poultry feed industries and farmers, if not checked. Therefore, the escalating price and seasonal fluctuation in the supply of conventional feed ingredients require alternative energy sources to be explored to ensure optimum performance of poultry at least cost [1].

Alternatives to conventional energy and protein feed ingredients for poultry must be cheap or cost-effective, readily available, have suitable nutrient composition and should have minimal negative effects on birds. Bearing this in mind, researchers, animal feed industries and poultry producers have over the years directed their attention towards finding alternative energy sources that meet the above-mentioned requirements [2–5]. One such alternative is cassava (*Manihot esculenta*). The nutrient composition of cassava differs according to the variety, the age of the harvested crop and soil and climatic conditions during cultivation. Generally, it is a staple/root crop tuber that is rich in carbohydrates, calcium, vitamins B and C and essential minerals and is considered to be a suitable alternative to corn as an energy source in poultry diets [6]. However, the use of cassava in poultry production is limited by its low protein content, unbalanced amino acid profile, dustiness and presence of anti-nutritional factors, e.g., cyanogenic glucoside. However, these shortcomings can be moderately remedied through adequate processing and use of feed additives. In 2016, the global production of cassava was estimated to be about 288.4 million tons [7]. The crop is grown in over 90 countries and is the third most important source of calories in the tropics, after rice and maize. It is a staple for half a billion people in Africa, Asia and Latin America.

Cassava is also a source of commercial animal feed. Over half of the cassava crop is grown in Africa, with a third in Asia and 14% in Latin America. Nigeria is the largest producer, growing 38 million metric tons in 2010. Other major producers are Brazil, Indonesia, Thailand, and the Democratic Republic of Congo [6]. The availability and the nutritional advantage of cassava make it a potential alternative to conventional maize in poultry feed. It is worthy to note that the use of cassava as poultry feed is not new. However, cassava root meal has not been fully adopted by the animal feed industry due to inconsistencies in animal response when it is included in diets. Over the years, various techniques, such as soaking, sun drying, boiling, ensilage and fermentation, have been employed to improve the nutritional value of cassava root meal with varying degrees of success achieved. However, none of these methods has successfully eliminated all the nutritional deficiencies inherent in this staple crop to make it possible for the root to be added at a 100% replacement of maize in poultry diet. Currently, cassava can only be added at levels of 30–40% in nutritionally balanced, pelleted diets. There is a great need to identify more effective processing techniques to improve the material for animal feeding [1]. In recent time, newer innovative processing methods such as the combination of various processing methods, application of genetic plant breeding techniques and the use of biotechnical methods have been tested in cassava processing.

Biotechnology is the use of living systems and organisms to develop or make products, or it involves any technological application that uses biological systems, living organisms or

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derivatives thereof to make or modify products or processes for specific use [8]. The use of biotechnical means to improve the feeding value of diets used for poultry is gradually gaining ground, and the results have been very encouraging so far. Research geared towards the improvement of cassava through biotechnological means has been reported by various authors. This chapter seeks to review the short falls and giant strides that have been achieved when cassava is processed with biotechnological methods as well as the effects of diets compounded with biotechnologically processed cassava meal when fed to poultry.

2. Nutritional composition of cassava

Information on the nutrient composition of cassava tuber is abundant. The variability of each major nutrient component is large. This variability is caused by several factors such as varieties (sweet or bitter varieties), geographical location, moisture content, processing technology and the age affects the chemical and nutritional composition of cassava root [9]. Various values for macro and micronutrients have been reported by various researchers as shown in **Table 1**. Cassava is high in carbohydrate; however, it has low protein and fat contents. The contents of some minerals and vitamins in cassava tuber are also low compared to the values reported in cereals and legumes [10].

2.1. Macronutrients found in cassava root

The main macronutrients found in cassava roots include carbohydrates, proteins, fat and fibre [11]. Raw cassava contains 60% water [12]. The carbohydrate content on dry weight basis has been reported by various authors to be within the range of 80–90% [13, 14], typically around 83.42–87.35%. On fresh weight basis, the carbohydrate content range reported by several authors is 32–35% [14] and as low as 29% [11]. Cassava root is rich in starch and digestible energy. The carbohydrate content of cassava is mostly made of starch of about 70–80% [15]. The starch in cassava root consists of two water-insoluble homoglucans, amylose (15–17%) and amylopectin (83%) [16, 17]. Compared to maize and barley, however, the starch content of cassava is lower. The percentage sucrose found in the bitter varieties of cassava has been reported to be lower than 17% sucrose found in the sweet varieties while small quantities of fructose and dextrose have been reported.

Cassava roots contain low levels of protein, with the protein content of both sweet and bitter varieties of cassava ranging from 1.17 to 5.13% [11, 17, 18]. These values are lower than those of cereal grains such as corn (8.8%) and wheat (11.3%) [19]. The crude protein content of cassava root consists of 50% whole protein, and the remaining portion consists of the free amino acids (predominantly glutamic and aspartic acids) and non-protein components such as nitrite, nitrate and cyanogenic compounds. The restricted use of cassava root meal in poultry feed formulation and feeding is due to its low protein content as well as a relative deficiency in essential amino acids [20]. Although high in arginine, glutamic acid and aspartic acid, other amino acids such as methionine, cysteine, phenylalanine, threonine, proline and isoleucine in cassava are low compared to maize [17]. The fibre content in cassava roots depends on the

Nutrient	Nutrient content per 100g	References
Macronutrients		
Water (g)	59.68	[11]
Energy (kcal)	160	[11]
Carbohydrates (g)	36.06	[11, 13, 14]
Protein (g)	1.36	[11, 17]
Total fat (g)	0.28	[11]
Crude fibre (g)	1.38–3.75	[11, 18]
Micro nutrients		
Vitamins		
Folates (μg)	27	[11]
Niacin (mg)	0.854	[11]
Riboflavin (mg)	0.048	[11]
Thiamin (mg)	0.087	[11]
Vitamin A (IU)	13	[11]
Vitamin C (mg)	20.6	[11]
Vitamin K (μg)	1.9	[11]
Minerals		
Sodium (mg)	14	[11]
Potassium (mg)	271	[11]
Calcium (mg)	16	[11, 18, 23]
Iron (mg)	0.27	[11]
Magnesium (mg)	21	[11]
Manganese (mg)	3	[13]
Phosphorus (mg)	27	[11]
Zinc (mg)	0.34	[11]
Cyanogenic glucoside		
HCN (mg)	1.34–2.78	[18, 22, 24]

Table 1. Concentrations of nutrients in raw cassava root (*Manihot esculenta*).

variety and the age of the root. The fibre content of cassava root is 4.4% for the sweet variety and 4.60% for the bitter cassava variety compared to 7.3 and 2.7% reported for maize and wheat, respectively [10, 21]. The fibre content in cassava root depends on the age and variety of the cassava. As the cassava matures, the fibre content increases while the sweet cassava root

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tends to contain less fibre. The fat or lipid content of cassava is very low; on fresh weight basis, the lipid content of cassava roots ranges from 0.1 to 1.5% compared to maize and wheat that have lipid contents of 4.7 and 2.47%, respectively [11, 21].

2.2. Micronutrients in cassava root

Cassava root contains several micronutrients, including water-soluble and fat-soluble vitamins. Cassava contains low amounts of the valuable B-complex group of vitamins and most of these are lost during processing. Compared to maize, the vitamin content in cassava root is low. Cassava root also contains substantial amounts of some essential minerals such as calcium, iron, potassium, magnesium, copper, zinc and manganese. Cassava root has been reported to contain (values in 100 g) 27 μg folate, 0.854 mg niacin, 0.048 mg riboflavin, 0.087 mg thiamin, 13 IU vitamin A, 20.6 mg vitamin C, 0.088 mg vitamin B6 and 1.9 μg vitamin K. The mineral content of cassava root compared to maize is usually low, except potassium (271 mg). The mineral content in cassava root includes calcium (16 mg), magnesium (21 mg), phosphorus (27 mg), sodium (14 mg), iron (0.27 mg) and zinc (0.34 mg) [11, 17].

3. Anti-nutritional factors in cassava root

Depending on the amount ingested, cassava contains anti-nutrients that can have adverse effects on health, especially when raw. The range of anti-nutritional factors found in cassava root includes cyanide (46–65 mg/kg), phytate (2160–3040 mg/kg), oxalate (2200–4400 mg/kg), tannins (40–60 mg/kg), saponin, trypsin inhibitors (100–400 mg/kg) and total alkaloids (10–52 mg/kg) [25]. Except for cyanide, the levels of these anti-nutrients in some varieties are below the normal toxic level [25]. Cassava variety and the amount of moisture it contains are major factors that determine the concentration of HCN contained in the root. The cyanogenic potential of known cassava cultivars ranges from 10 mg HCN/kg to more than 500 mg HCN/kg [26]. The bitter varieties have been reported to contain higher concentrations of HCN, whereas the sweet varieties contain lower levels of HCN [23]. The use of cassava as food and feed for human and animals is greatly compromised by its level of HCN. Cassava toxicity may be acute and/or chronic. Acute toxicity results from ingestion of a lethal dose and death is caused by the inhibition of cytochrome oxidase of the respiratory chain by cyanide [27].

The performance of broilers, and layer production and egg quality are negatively affected by cyanide levels of 100 mg HCN/kg and 25 mg HCN/kg, respectively [28]. The World Health Organisation set the safe level of cyanogen in cassava flour at 10 ppm [29]. When the cassava root is bruised or during the root peeling and grinding, two cyanogenic glucosides, linamarin (93%) and lotaustralin (7%), present in the cassava and synthesised from amino acids, isoleucine and valine, are hydrolyzed by an enzyme, linamerase, thereby releasing HCN [30]. When such cassava is ingested by human or animals, an enzyme, β-glucosidase,

produced by intestinal microorganisms, converts the HCN to hydrocyanic acid [31], which is toxic to both humans and animals. Acute cyanide intoxication, which results from excess cyanide residue caused by ingesting unprocessed cassava, has been reported to be associated with ataxia (a neurological disorder that affects the ability to walk). In an attempt to reduce or eliminate the toxic effect of hydrocyanic acid produced in the system, the liver and the red blood cells produce two enzymes, thiosulphate cyanide sulphur transferase (rhodanase) and mercaptopyruvate cyanide sulphur transferase, respectively. These enzymes are derived mainly from sulphur-containing amino acids (cysteine, cystine and methionine). With the influence of vitamin B_{12} (hydroxycobalamin), thiosulphate cyanide sulphur transferase (rhodanase) and mercaptopyruvate cyanide sulphur transferase help to convert toxic hydrocyanic acid to a more harmless thiocyanate, which is then passed out of the body in urine [27, 32].

4. Physical limitation to the use of cassava meal

Apart from anti-nutritional factors and nutrient deficiencies inherent in raw and unprocessed cassava root, the physical characteristics of cassava root meal such as dustiness, poor pelleting quality and poor pigmentation tend to also limit the use of cassava as feed ingredient in animal diets. These physical limitations have been reported to reduce feed intake and affect body weight gain and feed conversion ratio, especially in poultry. Crop impaction and irritation in respiratory tract have also been observed in animals fed cassava-based diet that does not contain oil or fed as mash [33]. Dustiness of a cassava-based diet is usually related to the form at which the feed is presented to the animal. Mash feed containing high level of cassava meal tends to result in dusty feed. Issues of dustiness in cassava-based mash diet could be ameliorated through adequate pelleting, resulting to improved feed consumption and poultry performance. According to Hahn et al. [34], pelleting cassava-based diet results into a diet that is denser, uniform and less dusty. Pelleting decreases the bulkiness of cassava-based diets by about a third, thereby overcoming issues related to dustiness. However, in farms where pelleting equipment is lacking, strategies such as the addition of oil or molasses can be employed to reduce issues relating to dustiness in unpelleted poultry feed. Wet mashed feed can also be fed to the birds to prevent dustiness; however, wet mashed feed should not be stored for long to avoid contamination and spoilage. Another physical attribute that limits the use of cassava as feed ingredient for animals especially poultry is the issue of lack of pigmentation. Cassava root meal is white in colour (it does not have any pigmentation). Feeding high levels of cassava root meal to layers and broilers has been reported to result in light-coloured egg yolks and pale meat, respectively. These eggs and chicken meat have been reported to attract low price because of low consumer appeal. When a high level of cassava meal is to be used, leaf meal or other pigmentation agents should be added to the diet in order to improve the quality of the product. At least 30–50 g leaf meal per kg of poultry diet can be used to prevent issues relating to lack of pigmentation in cassava root meal. Leaf meals such as cassava leaves, sweet potato leaves, ipil-ipil (leucaena) leaves and young grass have been reported to be effective [35].

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5. Effect of feeding raw/unprocessed cassava to poultry

There are several reasons why it is important to process cassava before using it in feed for poultry. Apart from the challenge that cassava root begins to spoil from 2 days after harvest due to physiological changes and microbial activity, unless kept under special storage condition [36], fresh cassava peel (*M. esculenta* Crantz) contains phytates and large quantities of toxic cyanogenic glycosides [37]. Feeding of fresh cassava roots may lead to cyanide toxicity, depending on the cyanide content in the roots, and thus should be processed in order to reduce cyanogenic and phytate contents [38] before being used as feed for poultry. Cyanide content in excess of 100 mg/kg diet impairs broiler performance, while laying hens may be affected by levels as low as 25 mg/kg diet [39]. In a study with local chickens [40], it was reported that birds lost 2 g/day when feed fresh feed materials, including cassava roots and duckweed, and attributed the poor performance mostly to the presence of ANF in the fresh feed materials. When unprocessed (peeled and unpeeled cassava root meal) was fed to broilers, Akapo et al. [41] reported depressed feed intake, weight gain and feed-to-gain ratio, mostly with birds fed the unpeeled cassava root meal. The study concluded that up to 100 g/kg dietary inclusion of the meals poses a threat to growth and health status of broiler chicks [41].

6. Physical processing of cassava for poultry feeding

Various physical processing methods can be applied to cassava and affect the nutritional quality of ingredient and its components for use in poultry feeding. Drying is the most common physical processing method for cassava. However, the type of drying method used seems to depend on the final cassava product being made. For example, sun drying has been reported to be more effective in reducing or eradicating cyanide in cassava than oven drying because with the former method the cyanide is in contact with linamarase for a longer period [42], with about 90% of cyanide content eliminated by sun drying alone [43]. Soaking of cassava before cooking or fermenting [44] or boiling for up to 15 min [45] is also important process applied in order to reduce the cyanide content of cassava before use for poultry feeding.

Wet fermentation (soaking over 2–3 days) causes a reduction in starch content while increasing total soluble and reducing sugar levels within the first 36 h and 24, respectively [46]. To be able to replace conventional energy source like corn, a high starch yield by cassava is important. High starch-yielding cassava can be achieved by using suitable drying conditions of raw materials. Olomo and Ajibola [47] found that oven-drying chips and flour resulted in a higher starch yield compared to sun drying. Wet milling of dry cassava chips has been reported to cause greater crumbling of cell content, resulting in a large fraction of fine fibre, whereas dry milling of the chips to 200 mesh size resulted in flour with highly varying particle size comprising very fine and coarse materials. However, wet or dry milling of dried roots had little effect on the composition [48]. While composition is not affected, particle size is a key factor that affects feeding and performance in broiler chickens [49, 50]. However, it is worthy to note that a high yield of starch does not necessarily mean the starch would have the desired quality

for any specific application [51]. Researchers have also shown that blending different native starch sources can give rise to an extensive array of properties, possibly obviating the need for chemical processing and minimising undesired properties of gels of individual starches (e.g., excessive cohesiveness in cassava and exudate in yam and corn) [52, 53].

According to different authors [54, 55], because cassava root has high moisture content (62–68%) and easily fermentable carbohydrate, it may be suitable for silage. As silage, cassava roots have high dry matter content, crude protein, crude fibre and ash [56]. Similarly, cassava leaf has been improved for use in poultry feeding through ensiling, which has been found to be efficient in reducing cyanide content in cassava leaf meal by 62% [57], while improving its digestibility [58]. To partially mitigate the reduction in feed intake by poultry due to the bulkiness and dustiness of cassava products, Morgan and Choct [59] suggested that poultry diets based on cassava products should be further processed through pelleting or addition of molasses or fat to improve texture, while simultaneously supplying essential fatty acids. However, it is important to note that there is no one physical processing method for cassava that gives the best quality cassava product, rather a combination of different methods is recommended based on nutritional objectives and variety of cassava used.

7. Chemical processing of cassava for poultry feeding

There is scarcity of literature on chemical processing of cassava for poultry feeding. Previous studies on cassava starch focused on starch-hydrolyzing enzymes such as α-amylase, amyloglucosidase and pectinase to achieve maximum hydrolytic efficiency of about 98%, resulting in 160 g/L of total reducing sugar [60], although this seems not have a direct application in processing cassava or its products for poultry feeding. However, this processing method has potential for use in the poultry feed industry. Recently, Olanbiwoninu and Odunfa [61] hydrolysed cassava peel into fermentable sugars using organic acid pre-treatment before enzyme hydrolysis. Because cassava peel is high in cellulose and hemicellulose (34.4%), this form of pre-treatment may be important in aiding the initial breakdown process before enzymatic hydrolysis is applied for it to be used for poultry feeding.

8. Microbial processing of cassava for poultry feeding

In recent times, the use of biotechnology in the form of microbial process in the improvement of cassava or its component for use in poultry feeding has become common. A major microbial process is solid-state fermentation, which involves application of fungal cultures to further enrich some local cassava products. Studies have examined different species of *Bacillus* and *Aspergillus*, although a comparative study showed that cassava starch was less susceptible to alpha-amylase of *Bacillus subtilis* and amyloglucosidase of *Aspergillus niger* hydrolysis compared with corn starch [62]. However, *A. niger* possesses the ability to produce fibre-degrading enzymes (hemicellulases, hydrolases, pectinases), proteases, amylases, lipases and hydrolysed tannins as well as agents that can hydrolyse tannins [63]. A study was conducted

where *A. niger* was used to improve the nutritional quality of cassava peel meal in order to use it as a carbohydrate source for broiler chickens [64]. The findings showed that hydrolyzing cassava peal meal with *A. niger* reduced the influence of cyanide in the peel. Enzymatic hydrolysis of cell wall polysaccharides improves the detoxification of cassava roots [65], hence making it a non-toxic and possible candidate as an energy source in the production of feeds for broiler chickens. The fatty acid functional group of lauric and palmitic acids can be imparted to cassava starch by bacterial and fungal lipases [66, 67]. Fermentation with *A. niger* was shown to increase the hemicellulose and amylopectin contents and metabolizable energy value of unpeeled cassava root meal for birds [68], suggesting that fermenting fungal organisms are able to release digestive enzymes which pre-digest the substrate, hence increasing the availability of nutrients [69]. Higher crude protein content and increased starch digestibility were recorded by [70] after fermenting cassava pulp with *Aspergillus oryzae*.

Cassava chip is an energy source with low crude protein, which when fermented with yeast could increase from 1–3% to 30.4% crude protein [71]. To obtain cassava products with high protein content and a relatively balanced amino acid profile for use in poultry feeding, cassava

Amino acid	Quantity (mg/100 g)
Alanine	70.5
Arginine	5.0
Aspartic acid	69.9
Cystine	5.0
Glutamic acid	189.4
Glycine	52.7
Histidine	55.4
Hydroxylysine	5.0
Hydroxyproline	5.0
Isoleucine	130.8
Leucine	201.5
Lysine	481.1
Methionine	16.3
Phenylalanine	167.8
Proline	47.9
Serine	29.8
Threonine	21.0
Tryptophan	15.1
Tyrosine	87.2
Valine	92.6

Source: [73].

Table 2. Chemical composition of yeast-fermented cassava chip products.

chip or other forms of cassava roots could be successfully fermented with yeast (*Saccharomyces cerevisiae*) [72–74]. Furthermore, a recent research showed that protein in cassava chips can be enriched by yeast fermentation up to 47.5% crude protein, which can be used as a replacement for soybean meal [75]. Cassava chips fermented with yeast have been shown to have high levels of lysine, glutamic acid, leucine and phenylalanine (**Table 2**). In contrast to fermentation with yeast, fermentation of cassava products with fungal inoculum has been shown to result in about 42% reduction in total amino acids [68, 76].

Sourcing cassava from different genotypes and harvesting at different times do not affect their susceptibility to glucoamylase microbial enzyme digestion, suggesting that cassava product quality can remain unchanged irrespective of the selected cultivars and growth conditions [77]. However, further supplementation of fermented unpeeled cassava root meal with exogenous enzyme has been reported to increase metabolizable energy values, indicating possible breakdown of fibrous content by the enzyme to release more energy [68]. Hence, microbial processes could be used to improve the quality of cassava and its products for use in poultry feeding.

9. Improving the feeding value of cassava through processing

For many decades, various processing methods have been used to enhance the feeding value of cassava for human and poultry use. These methods are all geared towards eliminating various ANF such as hydrogen cyanide, phytate, saponin and alkaloids that are inherent in raw cassava [78]. These processing methods have also been utilised to improve the nutrient deficiencies as well as various physical limitations such as dustiness and lack of pigmentation that tend to reduce the performance and product quality and increase mortality when unprocessed cassava is used as food or feed ingredient. Cassava processing methods can be classified into traditional [79] and modern [80]. Traditional cassava processing methods used in improving the nutritional composition and reducing the anti-nutrient content include drying, boiling, parboiling/cooking, steaming, frying, roasting, addition of oil, molasses, leaf meal and application of natural fermentation processes. These processes result in HCN losses ranging from 25 to 98% [30, 81]. Modern methods of cassava processing include addition of feed additives such as nutrient supplementation with amino acids, vitamins and minerals; addition of pigmentation agents; pelleting; synthetic enzyme supplementation; microbial fermentation of cassava root and genetic modification of cassava plant [82].

9.1. Traditional processing methods

Traditional methods of cassava processing, such as drying, boiling, soaking and natural fermentation, have been used for many years to reduce or eliminate ANF inherent in cassava root, thereby improving the feeding value of cassava for humans and animals. Drying has been reported to improve the shelf life and also reduce several ANF present in cassava roots [83]. There are two types of cassava drying processes—sun drying and oven drying [84, 85]. Sun drying has been reported to effectively eliminate more HCN than oven-drying process

during cassava processing due to the prolonged contact time between linamarase and glucosides during the sun-drying process [73]. It has been reported that the combination of soaking followed by boiling tends to eliminate more HCN than single/individual action of soaking or boiling alone [79]. According to Nambisan [30], up to 80% of the glucosides are removed by boiling and sun drying, while only about 20% are eliminated by frying, baking and steaming. Reduction in total cyanogens is effected by enzymatic decomposition of cyanogenic glucosides and/or leaching of cyanogens in the water in which the roots are boiled, with liberation of volatile HCN. Nambisan [30] concluded that crushing and pounding fresh cassava roots, followed by sun drying eliminates as much as 95% of the cyanogens and is the most efficient traditional method used in reducing the ANF inherent in cassava root. Soaking of cassava chips in water for about 24 h prior to sun drying reduced the HCN in three cassava varieties from 108.37 to 10.83 ppm (reduced by 90%), from 66.45 to 13.33 ppm (reduced by 79.94%) and from 58.63 to 15.0 (reduced by 74.42%) [86]. The authors highlighted the importance of soaking of cassava chips for at least 24 h prior to sun drying for a safe level of HCN in the flour. Natural fermentation of cassava pulp for 96 h reduced the HCN by 22 ppm (52.4%). Soaking of the sliced cassava tissue for 24 h prior to sun drying resulted in 16 ppm (38.1%) and 15 ppm (38.4%) HCN reduction for two cassava varieties studied. The HCN loss during sun drying was 6 ppm (14.3%) and 5 ppm (12.8%) for the two cassava cultivars used in the trial [78].

9.2. Modern processing methods

With the established fact that cassava root is a rich energy source but has low protein, amino acid, mineral and vitamin contents and also contains ANF (most notably high cyanogen glucoside level), the need for adequate processing cannot be overemphasised. Traditional methods of processing, such as drying, have been used for many decades; however, results have been inconsistent and are not able to improve the nutrient composition and reduce the ANF content to below the United Nations' recommendation. This presents the need for more modern, efficacious and multi-targeted technologically driven approaches. In order to achieve safe levels of 10 μg CN/g in cassava products, new methods of processing, especially for cassava containing more than 250 μg CN eq./g, are needed [30]. In recent times, the use of biotechnological method of processing cassava aimed at improving the nutrient composition and reducing ANF inherent in the crop has received a lot of attention from several researchers. Biotechnology involves the use of living biological systems and organisms or derivatives to develop, modify or make products or processes for specific use [87]. The use of biotechnology (depending on the focus of its application) often cuts across other closely related fields such as bio-engineering, biomedical engineering, molecular engineering, bio-manufacturing and bioprocessing [87]. The use of bio-engineering, bioprocessing and bio-manufacturing techniques in crop improvement has been gaining a lot of research interest and is the main focus of this write-up. With reference to cassava plant, the use of biotechnology involves the exploitation of biological processes, especially the genetic manipulation of microorganisms for the production of hormones and enzymes, and manipulation of genes with the aim of producing disease-resistant varieties, improving the nutrient composition, as well as eliminating any ANF inherent in cassava crop. Several studies have shown the efficacy of biotechnologically targeted processing methods in improving the feed value of cassava for both humans and

animals, for instance, in a study to ascertain the feasibility of enhancing root cyanide assimilation into protein, optimally overexpressed Arabidopsis CAS and NIT4 genes in cassava roots resulted in up to a 50% increase in root total amino acids and a 9% increase in root protein accumulation [88]. It has been hypothesised that cyanogen toxicity in cassava foods can be accelerated by cyanogenesis and cyanide volatilization during food processing [89]. To achieve this objective, the authors expressed the leaf-specific enzyme, hydroxynitrile lyase (HNL), in roots. This enzyme catalyses the breakdown of acetone cyanohydrin to cyanide. Expression of HNL in roots accelerated cyanogenesis by more than threefold, substantially reducing the accumulation of acetone cyanohydrin during processing thereby reducing the HCN content.

According to Siritunga and Sayre [89], cyanogen-free cultivars were generated by selective inhibition of CYP79D1/D2 gene expression. The CYP79D1/D2 enzymes catalyse the first dedicated step in cyanogen synthesis. Tissue-specific inhibition of CYP79D1/D2 expression in leaves leads to a 99% reduction in root cyanogen levels, indicating that the cyanogenic glycoside, linamarin, is synthesised in leaves and transported to roots. Zvauya and Muzondo [81] concluded that there was a marked improvement in protein level following microbial fermentation of cassava with *A. oryzae*. There was a 13.5% increase in protein and a marked reduction in HCN after cassava root pulp was fermented by *S. cerevisiae* in solid-liquid media fermentation conditions during 132 h and dried at 30°C [71].

A combination of microbial fermentation and other processing methods such as sun drying and milling led to a decrease in the total cyanogen level by 40% (158 mg/kg dry weight to 54.2 mg/kg dry weight). Oboh and Akindahunsi [90] reported that protein content of cassava flour improved when *S. cerevisiae* was added to raw and unprocessed cassava. The authors further observed that the cyanogen content also decreased. In an experiment aimed at enhancing starch production in cassava through genetic modification, Ihemere [91] reported an increase in carbohydrate/starch content in transgenic cassava due to enhanced root ADP-glucose pyrophosphorylase (AGPase). Leyva-Guerrero [92] reported that a biotechnological program known as BioCassava Plus employed modern biotechnologies to produce novel cassava germplasm with increased nutrient levels. The program demonstrated that cyanogens play a central role in cassava nitrogen metabolism and that strategies employed to increase root protein levels result in reduced cyanogen levels in roots. Furthermore, the program also demonstrated that enhancing root iron uptake has an impact on the expression of genes that regulate iron homeostasis in multiple tissues. These observations demonstrate the complex metabolic interactions involved in enhancing targeted nutrient levels in cassava plant. Available research results have demonstrated that micronutrient-enrichment traits are available within the genomes of these major staple food crops, including cassava that could allow for substantial increases in the levels of Fe, Zn and provitamin A carotenoids (as well as other nutrients and health-promoting factors) through biotechnological means without negatively impacting crop yield [93]. According to Refs. [46, 94], it was recommended that the target of biotechnological application in cassava processing should be the development of appropriate starter culture for cassava processing that will effectively produce linamarase enzymes for detoxifying cassava, break down starch to the simple sugars needed for acid production, improve the protein content of the products, reduce processing time and yield products with stable desired qualities. In addition, the authors concluded that *B. subtilis* produces amylase

and other enzymes that are necessary for the breakdown of starch to sugars, which are needed for the growth of other fermenting microorganisms. The process of increased proliferation of lactic acid bacteria leads to a corresponding increase in nutrients and a reduction in ANF in cassava root that has undergone microbial fermentation. Lactic acid bacteria convert cassava sugars to lactic and other acids that contribute to the flavour in addition to having preservative effects. Strains of *Lactobacillus plantarum* that is capable of producing amylase and linamarase have been used to improve starch hydrolysis and achieve a 98% cyanide detoxification of cassava. Linamerase obtained from *Lactobacillus delbrueckii* NRRL B-763 when added to raw cassava tuber resulted in a 95% reduction of HCN [95].

9.3. Amino acid supplements

As cassava products are low in protein and/or deficient in amino acids [96–98], supplementation of amino acids, especially methionine and lysine, has been reported to be a viable method for improving the quality of diets containing cassava and its products in poultry feeding [99]. It has been reported that cyanide can be detoxified to thiocyanate by the enzyme, rhodanase, with methionine as the sulphur donor [100], which makes this amino acid a limiting factor in cassava-based diets. In addition to boosting amino acid content of such diets, an extra dose of methionine may contribute to cyanide detoxification [43]. A study on the effect of levels of methionine supplementation (0.2 and 0.4%) in cassava peel-based diets for broilers concluded that up to 15% cassava peel meal can be substituted in broiler diets using 0.2% methionine, while up to 20% cassava peel meal inclusion requires 0.4% methionine supplementation for birds to achieve the desired productive performance in terms of weight gain and feed conversion [101].

9.4. Miscellaneous supplements

The scope for improving the utilisation of cassava products is wide. Researchers have attempted to use various supplementations other than enzymes and amino acids to enhance the use of cassava products for poultry feeding. For example, feeding cassava chips supplemented with *Moringa oleifera* leaf meal at 5 and 10% levels enabled the chips to replace maize at 55.56 and 83.33% in the diets of broilers, with no negative effect on productivity and blood function when 5% *M. oleifera* leaf meal was added [102]. Furthermore, Tesfaye et al. [103] tested cassava root chips supplemented with *M. oleifera* in layers. They reported that body weight gain, egg weight and hatchability were higher when birds were fed diets containing up to 50% cassava root chips as a full substitute for corn, supplemented with 5% *M. oleifera*. *Moringa oleifera* has high levels of vitamins and minerals and a rich amino acid profile [104], hence supplementing it in diet containing low-protein cassava product may enhance the nutritional value of the diet. Again, because cassava root meal is deficient in carotene and other carotenoids, one of the ways to overcome this deficiency is to supplement cassava root meal with cassava leaf meal with its high carotene and protein, hence combining a high-energy/low protein and high protein/low energy ingredients to meet the nutritional requirement of birds. In a study where maize was replaced with diets containing varying levels of cassava root meal and cassava leaf meal mixture showed that feed intake was improved, mortality was low and feed cost per weight gain was least when broilers were fed up to 75% of such mixture in the diet [23]. Due to dustiness,

researchers have supplemented cassava meal-based diets with oil to reduce this limitation; while in some cases, palm oil supplementation is used to balance energy in the diet. However, Ukachukwu [105], in a study involving composite cassava meal supplemented with or without palm oil and/or methionine, reported that such supplementations increased body weight and feed intake, while improving feed conversion. In another study by Kana et al. [106], they concluded that cassava meal with 3% palm oil and 1% cocoa husk supplementation can replace maize up to 75% in the diets for broilers without any adverse effect on bird performance.

10. Conclusion

There is an increasing demand on food and feed resources by man due to rising global population. To avoid future food crisis and loss of animal protein, animal nutritionists have continued to explore alternative feed resources to meet the needs of both man and farm animals. Several crops stand out as possible alternatives for feeding poultry. One such crop is cassava, a very abundant crop in tropical regions of Africa, Asia and South America. However, the use of cassava and its products is limited due to several reasons—short shelf life, low protein and amino acid contents, presence of cyanide and other toxic substances. To maximise the use of cassava and its products for poultry feeding, the application of different biotechnological techniques is needed, in order to enhance its preservation, improve its nutritional value and improve its utilisation in the poultry feed industry. In recent years, several researchers have used many of these techniques, ranging from different processing methods to supplementations with different feed additives. While there are no consistent agreements in some of the techniques, most of them hold promise for increased utilisation of cassava and its products with careful application of these techniques. It is necessary to continue to explore other methods and their applications so as to sustain the progress achieved in the biotechnological improvement of cassava usage in poultry feeding.

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