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Doctorate (PhD) Research Thesis

Improving Bovine Productivity in Central Africa: The case of Goudali Zebu Cattle under Ranching Conditions in Western Highland Sudan-Savannah of Cameroon

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DEDICATION

To all livestock farmers striving
for an environmentally-sound
Improvement of livestock
agriculture in Central Africa

Summary

The Central Africa bloc of nations' cattle industry needs to be developed. One essential input ingredient identified by stakeholders is the need for a constant supply of seedstock cattle of good quantity and quality and sustainable nutrition. Given the current orientation towards a boost in production that can meet up local demands as well as generate beef export among member states; the need for adaptable breeds with much more specific information on beef products derived from the different systems of husbandry is emerging.

Prevailing cattle production systems are muzzled by scarcity of affordable protein feeds. In this study we explored the suitability of use of low-toxic *Jatropha curcas* as a novel ruminant protein feed integrator and assessed the beef production performance of autochthonous Zebu Goudali beef-type cattle crossbred with the Italian Simmental and attempted an evaluation of the two cattle genotypes for prevalence and dermatophillosis-resistance allele markers under low input system of the Western highlands plateau Savannah of Cameroon; a cattle hotspot in the Central Africa.

There is a copious amount of information on the suitable level of introgression of exotic inheritance on indigenous cattle in low input production systems as a strategy to improve on their productivity. These studies indicate that; milk production, reproduction, growth performance and milk composition traits were all in favour of the 50% exotic cross. On behalf of FAO, Cunningham and Syrstad (1987) made an extensive analysis of results from crossbreeding in the tropics. Their clear conclusion was that consistent improvements in most performance traits were achieved in 'upgrading' cattle to as much as 50% with temperate breeds even though a smaller amount of study showed levels of introgression greater than 50% to be more important. A study in Brazil, Madalena *et al.* (1990), supports these findings in general, but found the 62.5% levelto be optimal. Results may, however, vary according to environmental conditions and traits studied. More recent studies involving meta-analysis of increasingly large numbers of results from the literature (McDowell *et al.* 1996; Syrstad, 1996; and Rege, 1998) as well as analysis of individual long-term studies in Asia (Jadhav *et al*., 199l), Africa (Rege *et al*., 1994; Thorpe *et al*., 1993) and Latin America (Madalena *et al.*, 1990) have confirmed the previous results. A general conclusion is, though, that crossbreeding to produce animals with up to 50% of the genes from temperate breeds can be recommended where crossbreeding is an option for genetic improvement. Crosses with less than 50% *B. taurus* genes have been found to be poor dairy animals (Syrstad, 1992).

Our investigation indicated that feeding *Jatropha curcas* cake up to levels of 4 mg of phorbol ester/day do not pose any evidence of pathology to small ruminants. Inclusions beyong 6 mg/day in the diet can pose deleterious health effects to the animals. Thus validating the potential role of *Jatropha curcas* protein integrator as an alternative and affordable protein source for ruminant nutrition.

On the crossbreeding component of our studies rather than looking at the impact of level of introgression of the Italian Simmental blood on autochthonous Goudali Zebu cattle; we devoted the major part of it to assessing under the Western Highland Plateau Savannah explored genetic markers of adaptability of autochthonous cattle breed and their 50% bloodline crossbred with the Italian Simmental to *Dermatophilus congolensis*, and the production performance values of their carcass and meat characteristics; in a context where these information are scanty. We observed that thoroughbred Goudali (G) young bulls show much lower *in vivo* and at slaughter performance than their crosses with Italian Simmental breed (SG) and that clinical dermatophilosis is common among G and SG cattle in the western highland plateau savannah of Central Africa and resistance cannot be predicted solely on the variability of BoLA-DRB3 gene. In addition, the results highlight the potential of optimising heterosis and maternal effects by improving bull's transfer and pre-slaughter conditions.

The study, within a broader whole supply value chain approach, validates the relationship between low-toxic *Jatropha curcas* cake, environmental adaptability; with respect to endemic dermatophilosis and livestock productivity improvement. This, considering the lowering effect on sector environmental footprints, increasing its feedstock flexibility and security while improving the social acceptance of both *Jatropha curcas* cultivation and intensive animal breeding as well as invoking the need for an increase in the level of animal productivity and welfare along the beef production value chain in Central Africa.

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CHAPTER ONE

INTRODUCTION

1.1. The Central Africa Sub Region

The Central Africa Region is a vast territory covering about 4,077,526 km^2 geo-spatially. It includes Cameroon (CM), Central African Republic (CAR), Democratic Republic of the Congo (DRC), Republic of the Congo, Chad, Gabon, Equatorial Guinea and Soa Tome and Principe (UN, 2013). The equator passes through it leaving one part above and the other below. This situation confers the region with a diversity of three principal climatic zones; humid, sub humid, dry and arid. The humid zone with Congolese and Guinean wetlands shades covers the southern parts of Cameroon and the CAR, the northern and centre CDR, Congo, Gabon, Equatorial Guinea and Sao Tome and Principe. The Sub-humid dry with Sudan and Sahel wetlands covers the north of Cameroon, the South of Chad, the centre and north of CAR, and the south of DRC. The arid zone covers the north of Chad. The average annual rainfall range from 400-1,500 mm.

Central Africa is dominated by forest and inhabited by tens of millions of people and currently holds an estimated 120 million inhabitants (UN, 2011). The different national population figures vary widely with the DRC inhabited by half of the total population. The population growth rate is relatively high but largely inferior to those indicated by other African bloc of nations. It stands at about 2.5%, with a wide disparity between the member states. Whereas 85% of the DRC's population lives in the forest, the situation is different in other countries where the majority is urban. The urbanisation rate is estimated at 42.85% with very highly urbanised states as high as 65% in Congo and Gabon. This urbanisation is accelerated both by natural population growth as well as rural exodus and migration (influx of persons from neighbouring countries as well as west Africa as seen in Angola and Equatorial Guinea) taking advantage of petroleum resources. The sub-region holds more than 60% of the African biodiversity and is classed first among African bloc of nations for its richness in a wide range of taxonomic species (White, 2000).

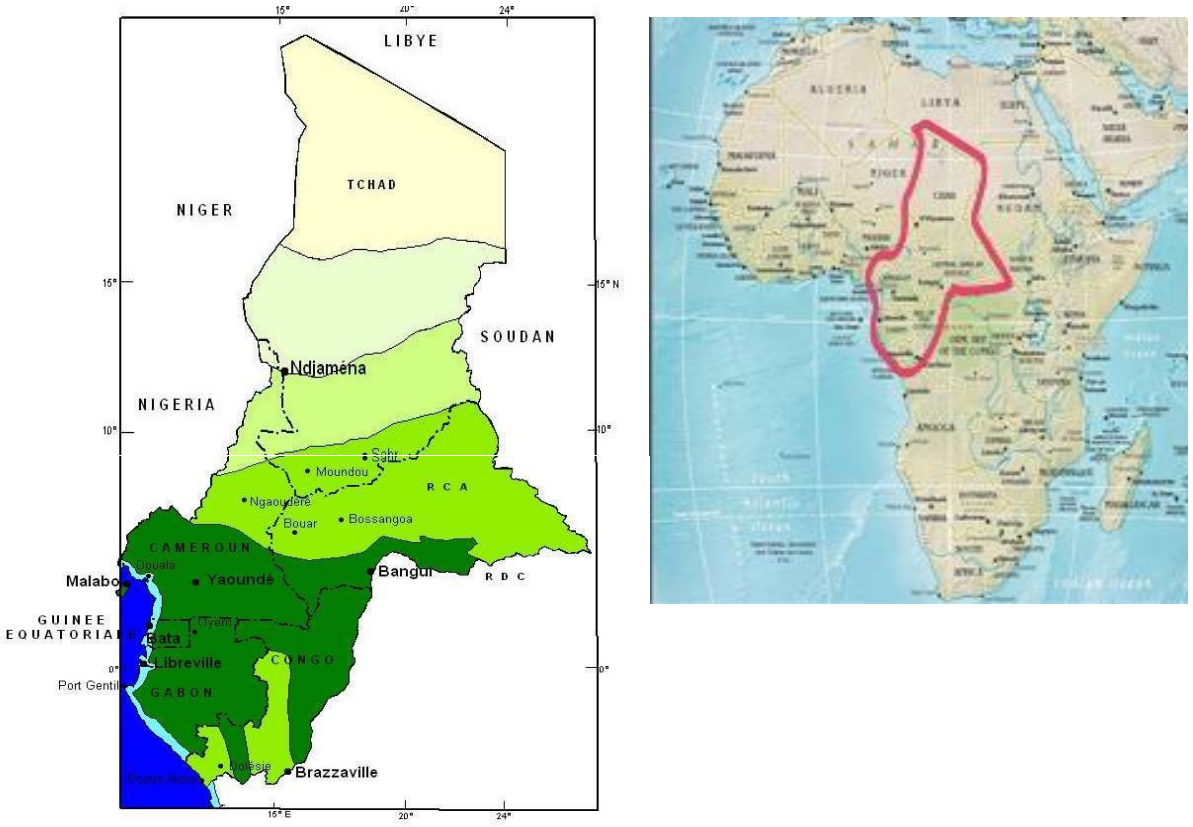

Figure 1. Map of the Central Africa Sub Region.
http://www.mapsofworld.com/africa/regions/central-africa-map.html

The economies of the Central Africa sub region share a number of distinctive structural traits, including high dependence on oil and forestry, volatile economic growth, weak intra-regional linkages, lack of transportation infrastructure, political instability and security problems, juxtaposition of wealthy coastal and poorer landlocked economies. Two countries; Cameroon and Gabon, whose economies account for more than two-thirds of the region's GDP, economically dominate the sub region. Moreover, with an estimated 2005 population of 30 million, CEMAC's six member countries depend on imported food, largely owing to the region's low agriculture productivity and the large number of people moving from rural to urban areas. As also identified in Zafar and Kubota (2003), from a trade perspective, the CEMAC zone is characterized by:

High dependence on oil and forestry. Offshore oil extraction is particularly important in the region, and especially in the Congolese economy. In Congo, oil exports account for about 90 percent of total exports, followed by wood exports, which account for about 8 percent of total exports. This high dependence on natural resource exports could lead to mistakenly conclude

CEMAC countries lack economic complementarities. The development of the non- oil sector should, however, bring forward underlying complementarities that permit enhancing trade in the region.

Limited intraregional trade. CEMAC's intra-regional trade is relatively low (an estimated 3 percent of total trade as opposed to about 9.4 percent for West African Economic and Monetary Union, though it is close to the levels predicted by the standard gravity models other control for economic size and distance (Masson and Pattillo 2005). Trade between CEMAC and WAEMU is almost nonexistent, while trade between CEMAC and France is more than 10 times CEMAC's intraregional trade.

Lack of economic complementarities. Most CEMAC economies share similar structural characteristics and undiversified production structures. Exports tend to be dominated by a few primary products, mainly natural resources: Congo, Equatorial Guinea, and Gabon primarily export oil; the Central African Republic exports diamonds; and Chad exports oil and cotton. The lack of diversification stems from poor infrastructure, a weak banking system, nontransparent trade policies, social instability, and other factors.

High tariff and nontariff barriers. A broad gamut of policies in place is thwarting trade, especially with neighboring markets. Traditionally, the CEMAC markets have been sheltered from competition with high tariff and nontariff barriers in all sectors other than unprocessed raw materials. Political and administrative obstacles, as well as poor transportation and telecommunications infrastructure, have reinforced market segmentation and further hindered regional trade integration. Moreover, CEMAC's trade structure resembles a "hub and spoke" arrangement, in which France is the hub and the CEMAC economies are the spokes, with weak intra-regional linkages.

Factor mobility is de facto minimal. Despite wage differentials and absence of formal migration barriers, labor factor mobility remains negligible.Lack of a common CEMAC passport and visa rrequirements, high unemployment low, despite the region having a common currency and a regional institutional framework with a shared central bank (BEAC) and bank supervisor (COBAC), a common legal framework, and regional decision-making bodies (IMF, 2006).

According to Tollens (2010), except in CAR and Equatorial Guinea, the agricultural sector has a

dualistic character: large groups of smallholder family farms versus larger commercial plantations as seen in Cameroon, Gabon and the Democratic Republic of Congo.

Livestock keeping is the mainstay of the pastoral systems and a large number of keepers in rangeland-based systems are in the sub region are poor with respect to national poverty rate (Thornton *et al.*, 2003). Livestock keeping is beset by several bottle-necks, one of the most important of which is livestock diseases, particularly endemic diseases transmitted by vectors such as ticks and tsetse flies (Rushton *et al.*, 2002), other protozoan, viral, and bacterial diseases. Other important husbandry problems in the area include breed productivity and animal feeds and feeding.

1.2. The role of livestock agriculture in Central Africa

Animal husbandry is a relatively important economic activity in the sub-region contributing 10 and 16% of GDP in CAR and Chad respectively. Subsistence beef production accounts for the nutritional well being of rural households in Africa. Furthermore, trading in cattle as means of livelihood for pastoral communities has continued to be supported by a growing demand for beef in urban centres (Bingsheng, 1998; Mwacharo & Drucker, 2005). Raising beef cattle based on rangelandpastures to sustain or better the offtake numbers is expected to continue in sub-Saharan Africa (Jarrige, 1992). Eastern Africa has a relatively higher productivity levels in offtake compared to Central Africa. Most livestock are kept by herders in extensive systems in the arid and semi-arid lands and smallholders in subsistence-oriented mixed crop-livestock systems. The majority of the livestock owners are poor and not commercially oriented. There is need for improvement of this supply of beef for human consumption, especially for the local and regional populations.

Table 1. Situation of Agriculture in Central Africa.

Country	Agriculture as % of GDP (2009)	Rural population as % of total (2009)	Population economically active in agriculture (2008)	Population (2008)	Active population as % in agriculture (2008)
Cameroon	19.47	42.42	3,617,000	19,617,000	18.95
CAR	55.51	61.26	1,263,000	4,339,263	29.11
Chad	13.63	72.86	2,960,000	10,913,667	27.12
Congo Rep.	4.51	38.28	489,000	3,615,152	13.53
DRC	42.91	65.42	14,098,000	64,256,635	21.94
Equatorial Guinea	3.46	60.46	167,000	659,197	25.33
Gabon	5.06	14.48	189,000	1,448,159	13.05
Sao Tome & Principe	16.81	38.62	31,000	160,174	19.35

Source: CEBEVIRHA, 2014.

Livestock products usually consumed includes meat, milk products, eggs, and to a lower extent skins (as seen in the South, western and central parts of Cameroon). Two principal groups of countries can be clearly delineated in Central Africa as producers and importers of animal products; Chad, Cameroon, and the CAR are the main livestock producers. These countries have natural vast afro-climatic zones favourable for pastoral low input-type husbandry activities, with their populations having a strong tradition for livestock husbandry. The second group is made up of five states, which have much smaller livestock populations and characterised by a weak tradition for animal husbandry with most of the agro-climatic zones not favourable for low input type of livestock farming.

Trans-boundary livestock movement is important and practiced as a way of life in pastoral milieu that gives access to natural biomes and water resources and trade networks (Bessong *et al.*, 2012) The existing patterns are dictated by geo-climatic and sociocultural realities in the sub region. Other factors influencing mobility are lack of water especially during the dry season, floods, and abandonment of zones infected/infested by disease/parasites, inter-ethnic conflicts and theft. Inter-community exchange of cattle even though of age, has remained mostly informal and weak in amplitude. Chad and CAR presents as net exporters of cattle in the subregion. Exchange of frozen meat is much reduced.

The entire sub-subsaharan Africa is known to suffer most from acute food insecurity among all regions in the globe. This hunger stricken population resides for the most part in the rural zones of the various countries where they survive by practicing subsistence agriculture, livestock farming, crop culture and fishing. Husbandry is essentially extensive with weak productivity representing sometimes a source of revenue and capital reserve for breeders. Meat production is projected to be increasingly largely insufficient to meet up with population demand as such fisheries products and game sometimes constitute the only source of animal protein.

Animal breeding programs consisting systematic set-ups of sound selection procedures in the process of influencing genetic change in Animals are completely absent in the subregion.

Table 2. Principal animals produced in Central Africa.

Evolution of Livestock Population (X 1000) in the subregion.

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Bovin	15,795	16,209	16,547	10,353	17,103	17,345	16,464	16,729	16,080	17,170	17,476
Sheep/ goats	24,841	17,152	25,609	24,014	26,141	26,689	27,726	28,387	28,503	28,921	29,277
Pigs	3,233	3,212	3,327	3,404	3,471	3,571	3,711	3,902	4,462	4,674	4,173
Poul-try	62,658	58,063	63,630	67,903	71,263	74,638	78,643	79,446	78,430	80,634	83,513
Camel	1,151	1,012	1,221	1,257	1,259	1,361	1,374	1,374	1,449	1,492	1,532

Source: CEBEVIRHA, 2014.

Table 3. Percentage contribution of livestock population 2002-2012.

		Bovine	*Sheep/goat*	*Porcine*	*Poultry*	*Camelid*
Chad	2002	38.85	32.04	2.07	7.98	100
	2012	44.51	34.45	2.64	6.81	100
Cameroon	2002	33.01	33.01	41.75	49.47	0.00
	2012	28.61	29.38	40.49	56.28	0.00
CAR	2002	20.76	12.75	22.57	7.30	0.00
	2012	22.54	18.27	28.88	11.13	0.00
DRC	2002	4.81	19.73	29.47	31.27	0.00
	2012	4.15	17.46	24.21	24.82	0.00
Congo	2002	0.06	1.58	1.76	3.40	0.00
	2012	0.14	0.32	1.68	0.48	0.00
Gabon	2002	0.03	0.90	2.16	0.56	0.00
	2012	0.03	0.12	1.92	0.48	0.00
Equatorial Guinea	2002	0.04	0.00	0.00	0.01	0.00
	2012	0.03	0.00	0.00	0.01	0.00
Sao Tome & Principe	2002	0.00	0.00	0.07	0.00	0.00
	2012	0.00	0.00	0.06	0.00	0.00

Source: CEBEVIRHA, 2014.

The production trend for the period 2002-2012 show annual growth rates of 12.17%, 2.06%, 6.10% and 5.95% for beef, mouton/chevron, pork and chicken. A value of 1.06% and 1.44% was observed for dairy and egg respectively. During the last two decades, the beef consumption has tripled while milk consumption doubled compared to to production figures from developing countries.

Several European breeds have been introduced in the DRC. Amongst these include Limousine, Shorthorn, Charolais, Hereford, Belgian Blue Belge, Santa gertrudis, brahaman, etc. Beef-type zebu of Indian, Pakistanis, have also been in cattle improvement in Congo. Among these zebu includes Boran, Bosmaran, Nelore, Bhagnari, Tharparker, Angolan Dhari e.t.c. Dairy type cattle introduced in Congo are mostly European breeds such as holstein, Jersey, Guernsey, Swiss brown and Simmental. For adaptational reasons there has been the introduction of other breeds of Indian or Pakistanis especially the zebu Sahiwal and zebu Red Sindi.

The livestock farming sector provides the most important opportunity to the rural masses in the Sudano-sahelian regions by virtue of the diversity of the stock reared (cattle, sheep, goats, pig, poultry etc) and also due to the multiplicity of livestock-derived activities; feedlot, dairy production, and transformation of milk and beef. Of the different livestock reared, cattle production is the most important activity that brings wealth and social security to families and the state.

This situation has a potential for improvement if the activity is followed up and supported with modern techniques in a holistic value chain perspective. The dairy aspect of cattle production is the aspect that needs more attention considering is ramifying implications on the national and family incomes and at improving food security in the sub-region.

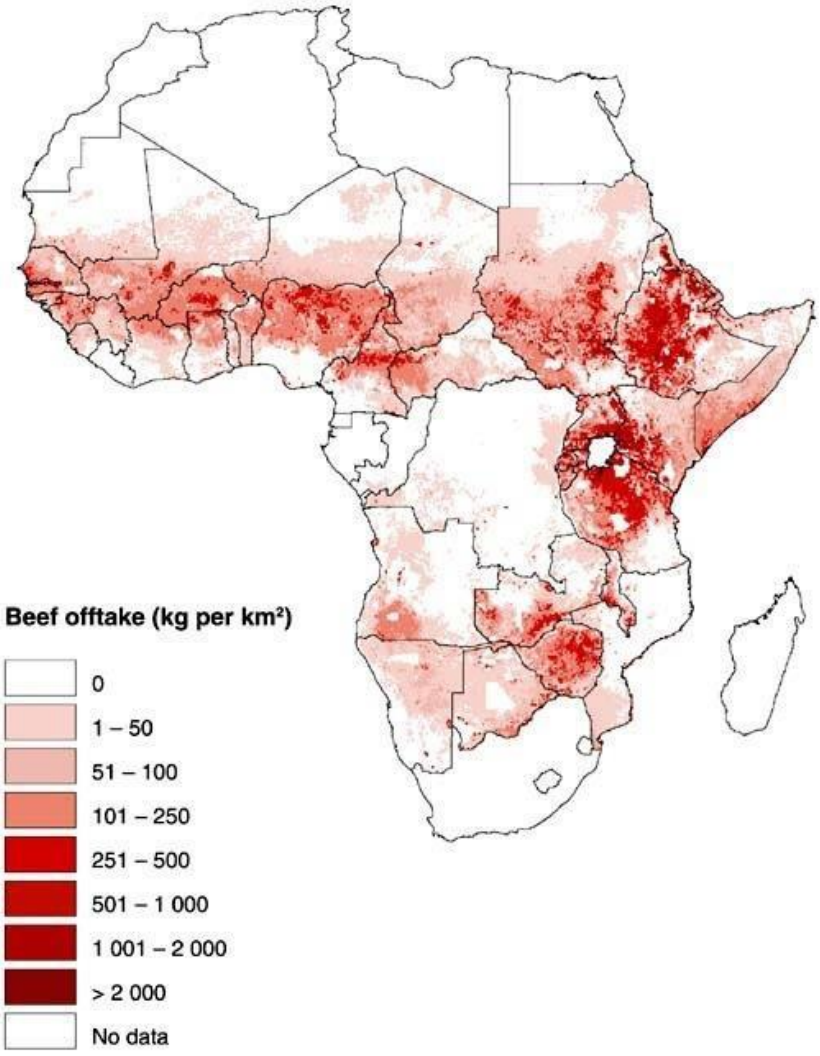

Figure 2. Estimated beef offtake in kg/km^2 from sub-Saharan Africa (FAO, 2002).

1.3. Cattle production environment and systems in Central Africa

The availability of biomass resource in Central Africa, which constitutes the base for cattle nutrition, determines directly the different approaches to cattle production. A major feature of the traditional pastoral production system is the seasonal transhumance herd migration between one geographical zone to another, in search of natural pasture, crop residues, water supplies and disease-free environment; and to avoidconflict with arable (crop) farmers. During the dry season, the movement is south-wards from the (arid and semi-arid) Sahel & Sudan savannah, towards the (subhumid) guinea andderived savanna. The movement pattern is reversed during the dry season, and seeks tomaximize use of scarce natural resources that are seasonally available. The movement cut across geographical, ecological and political boundaries.Along this reasoning, three main types of cattle stock farming systems can be delineated as follows: the extensive pastoral (traditional), agro-pastoral or semi-intensive, and intensive systems.

1.4. The principal cattle production zones in Central Africa

Central Africa has main cattle productions zones with a wide variety of breeds adapted to it as follows.

Zone I or the Sahara Zone. This zone is a vast desert territory of about 600,100 km^2 of the extreme north of Chad. It characterised by a Saharan climate made up of severe temperature fluctuations between diurnal (40^oC) and nocturnal (3^oC) and a weak annual rainfall (50 mm to 200 mm) which is observed mostly between July and August. It is the domain of dunes, barkans, and ergs where are found several oasis with green vegettion. Cattle breeding is essentially practiced around these oases with a total cattle population estimated at about 30,000 heads (Cebevirha, 2014).

Zone II or the Sahel Zone. This zone is immediately adjacent the Sahara zone at its southern extremeties with a surface area of about 571,600 km^2. It is caharacterised by a very long dry season (November to May) than the rainy season (June to October). The environment to the northern part is made up essentially of sand dunes, date palms, d'oueds and oases. Rainfall is about 400 mm per year. The southern part is covered by pseudo-steppes (*Cymbopogon, Aristida, Cenchrus and Setaria spp.*) and vast areas ocuppied by *Acacia* and *Zyziphus* where rainfall

is between 600 mm and 800 mm annually. This area represents the largest hotspot for cattle production in Central Africa; notably Kanem, Batha and Ouadai communities.

Zone III or the Sudan Zone. This area is located towards the end of the centre south of Chad and the extreme north and northern administrative regions of Cameroon, and the north-east of the Central African Republic.

It consists of about 550,000 km^2. The climate is characterised by relatively abundant rainfall which varies from 600 mm in the northern parts and 1,542 mm in the south. Maximum temperatures goes up to 37˚C in the southern part and 43˚C in the north with a more or less woody savannah in the Salamat cattle production basin, the mid Chari and the Logone areas and completed by a vast field of varied grassland in the north (*Adropogon gayanus* and *Pennisetum pedicellatum*), and to the south which is preponderantly by annuals (*Hyparrhenia rufa* and *Ctenium elegans*).

Sudan zone where acacia is found and the acacia forests of the north and the forests in the south passing through woody savannah towards the centre is a very important ruminant livestock zone especially for cattle. This particularty is so because of the rich natural pastures found in this locality which derives from its relatively higher rainfall.

Zone IV or the Guinea Savannah Zone. This is the guinea savannah zone which covers a total surface area of about 484,900 km^2. It is made up of two distinct parts; The Northern guinea savannah and the Western guinea savannah.

The northern guinea savannah covers the extreme southern parts of the Republic of Chad, the Adamawa and Northwest regions of the Republic of Cameroon and the Central African Republic where it ocuupies its central band from the borders with Cameroon to the borders with Sudan. The climate is typically tropical characterised by an average annual rainfall of more than 1000mm and a temperature fluctuating between 20˚C and 35˚C. The vegetation woody savannah with *Buthirospernum, Hymenocardia, Alizicarpus, Danielia* and *Lophira spp.* interspersed in vast tall grasses dominated by *Hyperrhnia, Uretrelum* and *Sporobolus spp*.

The Western guinea savannah in Central Africa is made up of two main bands; one covering the maritme facade of the Congo up to and beyond the borders with Gabon and the other extending from around Brazzaville in the southeastern part of Congo up to the borders with Gabon. It is truly a woody savannah with clear forest where *Panicum maximum*, *Andropogon gayanus* and *Pennisetum purpureum* grows abundantly and luxuriantly. The climate is humid

equatorial with a heavy average annual rainfall of 1,500 mm and temperatures relatively mild and varing between 24˚C and 30˚C.
The two sub areas described above offers relatively favorable conditions for cattle husbandry but requires strategies to mitigate the impact of the trypanosomes vector, tsetse flies.

Zone V or the Forest Zone

This zone covers almost one half of Central Africa; 1,014,000 km^2. It consists in:

- Cameroon, the Centre, South, Southwest, Littoral, and parts of East regions
- CAR, the extreme south starting from the Region of Bangui and Mobaye Regions
- Gabon, the entire national territory except the band of guinea savannah mentioned above
- Congo, equally the entire country with exception of the guinea savannah zone
- Equatorial Guinea, the entire continental region

The climate is equatorial characterised by heavy rainfalls of averaging 2000 mm per annum and a much relatively cool temperature varying between 20˚C and 30°C.

The forest zone is dominated by dense woody forests. The presence of tsetse flies is a constrain to cattle production. However, considering the quasi-permanent growth of a wide variety of grass types this zone typical to the Amazon has very high potentials for semi-intensive cattle production.

Table 4. Distribution of cattle Breeds in the different Agro-ecologic Zones in Central Africa.

Group	Breed	Agroecologic Zone					
		Sahara	***Sahel***	***Sudan***	***Nortwestern Guinea***	***Mid Guinea***	***Forest***
Zebu	Arab						
	Goudali						
	Djafoun						
	Akou						
	Toupouri						
Taurine	Kouri						
	Kapsiki						
	Namnchi						
	Ndama						
	Bakweri						
	Bakossi						
	Baoule						

Source: CEBEVIRHA, 2014

1.5 Cattle Farming Systems

1.5.1 Traditional (extensive) system

This system is characterised by a total dependence on native biomass and natural water sources for production. Indigenous breeds of beef-type cattle; notably, the Goudali, Aku or the Djafoun are grazed under control of herdsmen either on foot or horsebacks over extensive areas of natural pastures. Often times small ruminants, sheep.

The traditional extensive pastoral system is an economic activity based on a production system in which the pastoralist derive a major proportion of family's subsistence livelihood mainly from herds of domesticated cattle, sheep and goats, utilizing predominantly natural inputs. Migration with herds of animals across the various ecological zones is essencially in response to seasonal changes in weather, forage availability and disease situation. During the drier months (lasting up to 9 months in the arid zones), transhumant movements are from the arid and semi-arid zones of the north towards the tsetse fly infected sub-humid zones of the south, in search of pasture, crop residues, water and disease-free areas. This movement pattern is reversed during the dry season.

During the dry season, characterised by a paucity of water and poor pastures, parts or the entire herds of cattle may be moved to lower plains that bear relatively better quality pastures (transhumance). Loss of production is usually very high as a result to disease, parasites, malnutrition and theft.

Cattle are essentially a store of wealth and sold out to cattle traders or middlemen who channel them to be butchered for beef in crisis situations; such as ill-health, meeting-up with fines related to crop-farmer versus animal-grazer conflicts, or as gifts with social bearings such as marriages or death celebrations. Under this system milk, production is considered as a side-product rather than the primary objective for farming. Some cows with tolerable temperament are milked exclusively by hand squeezing and pulling of the teats and mammary glands and the milk is usually destined for consumption by the herdsmen and their families. Milk extraction is usually an activity for women and children. Excesses of milk collected in this system is boiled and sold as liquid milk or processed under artisan conditions into butter.

Inputs are minimal and essential elements in breeding that can engender genetic progress in the herds are lacking. Breeding values have no qualitative dimension neither are they defined. This farming system constitutes about 80-85% of cattle farming systems all over the national territory. An estimated 200,000 traditional cattle farmers practice this type of farming

1.5.2 Semi-intensive system

Semi-intensive systems are commonly found with farmers with an economic dimension to their production. Here, improved breeds of cattle (usually crossbreeds of local and improved varieties) are grazed on appropriate pastures, which may have been cultivated, and their feeding is supplemented with agro-industrial by-products such as rice bran, palm kernel cake, cotton seed cake, wheat bran, roasted seeds, maize offals e.t.c. This system is an improvement on the traditional extensive systems. This improvement permits the production of homogenous herds of animals with often time an improvement in the quality and quantity of milk and beef derived from them. Herd performance is in terms of inter-calving intervals and other records are usually kept.

1.5.3 Intensive husbandry system

Production farms with exclusively intensive production methods are a rarity in Central Africa. Production under zero grazing can be spotted in the Northwest and North regions mostly of imported breeds introduced by SODEPA, Heifer Project International (HPI), TADU Dairy cooperative and other private operators with institutional support (public, research among others). These farms have animals stock of improved varieties imported live to boost mostly dairy production for economic earns. The Holstein Friesian, Jersey and their crosses with the Goudali are the most common breeds encountered under this system.

Under this system, the animals are hardly displaced for any reason other than exercise. Feed supplementation with grains and other agro-industrial by products is significant. Cows are stall-fed and bred using imported semen of various types. In 2014, SODEPA semi-intensive production launched the first Multiple Ovulation and Embryo Transfer (MOET) programme in the country with the application calf sex-influencing agents to boost the production of high performance genetic stock at the Jakiri Livestock Station. The productivity of these farms are outstanding compared to those of the other systems. However, the system is labour intensive. The number of farms under this system is quite small and rarely up to 1% of the total national herd size. Lack, and cost of appropriate machinery related to dairy farms are important limiting factors to this method of production

1.5.4 Ranching

Ranching as practiced in Central Africa is essentially an extensive form of grazing on natural pastures but within the confines of a limited perimeter. Production characteristic of ranching do not move stock on transhumance. Production is economy driven and sales of finished products such as replacer heifers, finished young bulls for butchery, are done at well defined ages of the stock. Pasture maintenance and management, pasture improvement, zootechnical infrastructures (Craals, animals restraint units, acaricide dips) road networks, and veterinary sanitary, prophylactic endemic disease programmes are well planned, structured and implemented to optimise production and gains. Examples of such farms include the SODEPA network of ranches (Dumbo, Faro, Ndokayo and Jakiri Livestock Station - with an estimated 19,000 heads of cattle), Elba Ranch, Heritage Ranch, Fadil Ranch, amongst others in Cameroon, In Cameroon, cattle ranches are mainly located in the North (>75%) and in the high altitude pastures of the Adamaoua plateau Western highlands (15%).

Cattle in the Congo Basin is essentially in the hands of large companies engaged in ranching operations over large surfaces. According to Mammerick (1986), it is in 1886 that imported cattle from Angola and the island of Madeira, arrived on the island of Mateba (estuary of the Congo river). This was actually the start of the first cattle breeding operation in DRC leading to several thousands of heads of cattle in 1900 known as Mateba island cattle. Later, given the presence of the tsetse fly, vector for the transmission of trypanosomosis, imports of West African cattle led to the multiplication of N'Dama breed in Bas-Congo especially by the JVL, a company that is currently part of Orgaman group, which also has operations in Bandudundu.

Cattle breeding is practiced also in several regions of DRC, particularly in Katanga (Grelco, Pastorale, Marungu, Kundelungu). Currently, the largest livestock company: Grelka (Grands Elevages de Katongola) of the Forrest Group (GFI) has 35,000 cattle of the Grelka breed of Afrikander origin and improved recently by Bonsmara cattle imported from South Africa. An experimental cross with Belgian Blue cattle was launched in 2008. Grelka operates in altitude on the Biano plateau and produces castrated bulls, sold at 4 years old. Cattle ranching is also observed in Gabon where the company SIAT continues crossing breeding of the N'dama, Senepol crosses and Zebu Goudali imported from Cameroon.

1.6. Challenges and perspectives of livestock agriculture in Central Africa

Sub-Saharan Africa as a whole is projected to bear the highest world regional food demand over the next 15 years by about 60%. Climate change is equally projected to reduce crop yields by 15 to 20% in this region if temperatures rise above two degrees Celsius (World Bank, 2015). A durable solution to offset food shortages is a conscious effort for food production systems to increasingly and simultaneously deliver higher agricultural productivity, greater cli- mate resilience, and reduced carbon emissions (World Bank Group, 2015).

Table 5. Projected trends in meat consumption from 2000-2050.

Region	**Production**			**Consumption per Capita**		
	1999-2001	Growth Rate 1999-2001 to 2030	Growth Rate 2030-2050	1999-2001	Growth Rate 1999-2001 to 2030	Growth Rate 2030 to 2050
	1000 tonnes p.a	% p.a	% p.a	kg p.a	% p.a	% p.a
Sub-Saharan Africa	5,564	3.3	2.8	9.5	1.2	1.4
Near East/North Africa	7,382	3.3	2.1	21.9	1.6	1.1
Latin Amer- ica and Car- ribbean	31,608	2.2	1.1	59.5	0.9	0.7
South Asia	7,662	3.9	2.5	5.5	2.7	1.9
East Asia	73,251	2.1	0.9	39.8	1.5	0.9
Developing World	125,466	2.4	1.3	26.7	1.2	0.7
World	229,713	1.7	1.0	37.6	0.7	0.5

Source: FAO, 2007. The State of the World's Animal Genetic Resource for Food and Ag- riculture; Edited by Barbara Rischkowsky and Dafydd Pilling.

The expected response to the food deficit situation calls for a general increase in the number of meat animals in the subregion, the need to mainstream suitable livestock resources and improving their genetic value, and an annual increase in production levels. Overall, growth originating from agriculture has been two to four times more effective at reducing poverty than growth originating from other sectors (World Bank, 2007).

The current global orientation for agricultural growth is that of improving productivity while ensuring sustainability through efforts that target the lifting out of poverty and hunger through sound environmental management. The food systems needs to help reduce the significant risks and burden of animal health, including zoonotic diseases. Climate change could increase risks and uncertainty by affecting the range, seasonality and incidence of animal diseases. Reducing livestock losses helps to preserve a critical capital asset and source of income and food for poor people.

Livestock keeping is the mainstay of the pastoral systems and a large number of keepers in rangeland-based systems are in the sub region are poor with respect to national poverty rate (Thornton *et al*., 2003). Pastoralists have increasingly become less food secure and vulnerable to poverty over the last two decades. This is primarily due to increasing human population and changes in land tenure systems as well as the harsh agro-climatic conditions associated with sub-saharan Africa (Rushton *et al*., 2002, Wollny, 2003).

Past investments, both from the private and public sectors, in the development of the livestock sector have been largely project-based and disjointed, with little regard to long-term institutional development. Moreover, private sector investments in the sector, which should drive accelerated and equitable growth, stimulate growth in other sectors and galvanize widespread socio-economic transformation, have been subdued by the lack of supportive policy environments, the lack of appropriate infrastructure and the non-availability of reliable supplies of essential inputs and services, thereby making the sector uncompetitive and thus unattractive to investors. With appropriate investment, the livestock sector stands to not only contribute significantly to accelerated economic growth but also to be a key driver for stimulating growth in other sectors, and for attaining the development goals of food and nutrition security, eliminating hunger, improving livelihoods and engendering resilience (AU-IBAR, 2014).

Notwithstanding, there are numerous examples of successful performance of the sector on the

continent. These include the thriving beef sector in countries such as Botswana, Namibia and Swaziland, the growth and development of the smallholder dairy sub-sector in Kenya, the export of live animals from the Greater Horn of Africa to the Middle East (Djibouti, Ethiopia, Somalia and Sudan), the cross-border mobility and pastoral resilience in the ECOWAS region, the water use and conservation in North Africa and the successful eradication of rinderpest from the continent. These cases provide encouragement that the transformation of the sector on the continent is feasible.

The expected response to the food deficit situation calls for a general increase in the number of meat animals in the sub-region, the need to mainstream suitable livestock resources and improving their genetic value, and an annual increase in production levels. Overall, growth originating from agriculture has been two to four times more effective at reducing poverty than growth originating from other sectors. The current global orientation for agricultural growth is that of improving productivity while ensuring sustainability through efforts that target the lifting out of poverty and hunger through sound environmental management. The food systems needs to help reduce the significant risks and burden of animal health, including zoonotic diseases. Climate change could increase risks and uncertainty by affecting the range, seasonality and incidence of animal diseases. Reducing livestock losses helps to preserve a critical capital asset and source of income and food for poor people.
Rapid population growth, urbanisation and associated changing lifestyles are currently fuelling an increase in demand for meat, milk and their products in Africa south of the Sahara as a whole. Ruminant livestock production holds a strong cultural value and represents veritable economic opportunities for Cattle contribute about 28% percent of total animal protein in the area.

Besides a widely diffused interest in cattle production as characterised in its intricate role in the livelihood of the population, production practices that favour breed improvement leaves much to be desired and the livestock sector contribution to the national economy is extremely low. The current high demand for beef products represents real opportunities for entrepreneurs who have the financial potential to impact an economic dimension to the current subsistence production. A major task which has been subject of several workshops is for government to provide an enabling environment for easy insertion of economic operators to invest in a modern livestock farming characteristic of those of emergent countries. Dearth of information on different breed performances and productivity poses a serious setback for economic considerations in

planning breeding and making projections required for financing production. Genetic improvement of indigenous cattle, basically focusing on crossbreeding under low input systems has been variously recommended. Plans aimed at improving indigenous cattle productions in the tropics have been discussed (Cunningham & Syrstad 1987; Dolberg 1991). Convergently, the use of nucleus herds in cattle improvement is proposed. Based on these ideas, commercial herds could be used as nucleus herds while base herds could be those in the traditional pastoral sector. This implies that well adapted improved heifers and bulls could be obtained from the commercial sector for subsequent improvement of village herds as discussed by Hicks (1991). The advantages associated with crossbreeding are a well-docu- mented phenomenon. Research has clearly and repeatedly demonstrated benefits associated with implementation of crossbred mating systems in various production systems. Generally crossbred animals out perform their straight bred contemporaries. Exploitation of this phenomenon in the Western highlands of the northwest of Cameroon is not of common place because context based studies in the environment are absent. This study seeks to determine and provide crossbreeding estimates between the Goudali breed and the template breed and evaluate the adaptability of the offspring in Seed stock multiplication stock farm in the locality in order to provide specific information related to the production potential of crossbred cattle in the environment innovation is context-specific challenge driven solution.

1.6.1. Nutritional challenge

Meeting consumer demand for more meat in the sub-region is dependent to a major extent on the availability of regular supplies of appropriate, cost-effective and safe animal feeds. As a matter of fact, few issues have generated as much public concern in recent times, however, as the protein supply in feeds of livestock production. The different cattle farming systems in existence in Central Africa can be directly linked to ready accessibility to feeds and water. The cost of animal feed inputs from sources such as maize and soybean are relatively high making their use in feed production unprofitable. This relative high cost of conventional protein feed sources such as soybean is related to their market value as direct human food stuff in the sub-region. Maize and soybean are very important foods for human nutrition. Besides, these crops are grown on the same agricultural lands with other food crops by the same source of labour. Considering the high demand for soybean for human consumption, the economics of their use in beef production is limited. With an agricultural system characterised by manual cultivation of cereals and other food crops, the amount of suitable protein energy feed integrators such as

soybean and maize produced is dismal and posses highly competitive market value; considering that these same products are direct staple foods for the human population.

To improve on cattle productivity, it is clear need for research on novel alternative protein feed sources for ruminant nutrition. While it is recognised that most of the additional supply of animal products may come from intensive poultry and pig production, cattle and small ruminants are capable of production on feeds that are high in complex carbohydrates and not usable in quantity by monogastrics. They offer considerable opportunities for meat and milk products in developing countries.

Jatropha curcas seed cake; a co-product generated in the process of oil extraction is a valuable animal feed after detoxification. It has a high energy content of 25 $MJkg^{-1}$ and is high in protein, 58.1% weight compared to soy meal, 48%. Unlike other major biofuel crops such as maize, soy and rape, Jatropha is not used for food production and can be grown in marginal and degraded lands where food crops cannot thrive well. As a crop that does not influence the production of cereals, it's cultivation using both traditional plant breeding and biotechnological techniques hold potentials for improving performance, reducing feed costs and thus making meat protein production more affordable.

Due to the increasing demand for beef in recent years, beef cattle are considered a strategic commodity in developing the livestock sub-sector. Unfortunately, national production of beef has never met the demand. Therefore, an effort with the goals of increasing production and productivity of beef cattle through the optimal use of local resources is strongly needed.
Improved cattle production could be achieved by maintaining and increasing the population as well as improving cattle performance, primarily through nutrition, reproduction, health and genetic aspects.

The majority of cattle are maintained extensively and traditionally, but farmers do not give enough primary attention to feed supply, either quantitatively or qualitatively. Therefore, the performance of cattle is considered to be decreasing.
Absence of a defined selection in most of Sub-Saharan Africa has coupled witha high demand and sectoral regulations of the trade and of livestock distribution, has seen heavy heaviest cattle have moved off the farm very quickly. This has tended to cause a negative selection. At the same time, the availability and regular use of the same bulls may cause inbreeding.

The effort to increase productivity of cattle in some areas made use of crossbreeding with exotic

breeds. However, this effort was apparently not followed by management improvements. Moreover, it may have negative impacts due to genotype– environmental interactions. Beef cattle that have superior genetic composition but do not get enough feed may not survive. On the other hand, indigenous cattle generally characterised by small body size have resistance to high environ- mental stress.

Therefore, there is a need to define real problems, determine facts, and try to find appropriate solutions. In reality, genetic and management problems need to be restructured, but which one is more important in terms of priority? Improvement must be sought by using appropriate innovative technology that can readily be applied and that is socially acceptable, economically feasible and environmentally friendly.

Breeding strategies to produce feeder cattle must be developed in order to reduce inbreeding and negative selection. AI activities need to be assessed and re-evaluated in terms of both the crossing pro- gram and the technology. Therefore, the breeding program must be directed to making use of the genetic potential of cattle, primarily its adaptation and its fertility as well as its ability to produce good carcass quality.

Raising beef cattle for breeding purposes to produce feeder cattle may not be profitable in partial budget analysis. However, if the raising of animals could be integrated with other agricultural production systems, it might have good synergism. It is also hoped that the Crop–Livestock System (CLS) can ease management problems, primarily by improving the supply of good quality feed.

1.6.2 Bovine Genetic Improvement

From a broad based knowledge perspective cattle breeding and genetics in Central Africa is yet to be formally organised into breed specific farms with clear-cut breeding objectives that put in evidence expected progeny differences within the framework of selection schemes as seen in some breeds in North, southern, and Eastern African countries. Examples of this are the cases of Boran and Holstein breeds of cattle in Kenya and Ethiopia.

For selection schemes that can make genetic progress amongst indigenous breeds in Central Africa to be developed. There is a backlog of actions ranging from sensitisation/organisation of breed-specific farmers organisations through policy, to executing institutional frameworks and product commercial needs.

In the absence of organised straight breeding schemes in which genetic progress of the various breeds are managed and enforced in a mutually coordinated manner by all stake holders, the use of crossbreeding options as a short term strategy to improve production is widely practiced in small scales and in an isolated fashion by farmers who seek to boost the productivity of their herds. For crossbreeding to be sound, information on the relative performances of breeds and their crosses, especially under varying environmental conditions and factors affecting them, is needed. In Central Africa, there are limited reports on growth performance of B. taurus x B. indicus crosses and on estimates of genetic parameters for growth traits as demonstrated by works on breeds in other regions of Africa (Banjaw & Haile-Mariam, 1994; Demeke *et al*., 2003). Additionally, accurate estimation of breed additive and non-additive effects and separation into their causal components is essential for the design of a breeding programme which fully exploits the value of cross- breeding (Cunningham & Syrstad, 1987).

Breeding and genetic management is an essential part of operational decision making, with decisions notably impacting profitability. Potential commercial cattle producers are likely to input cost management decisions in project planning. In the same vein, they must decide on practices that affect productivity and returns. A well-designed and implemented crossbreeding system in commercial cattle operations is one proven way to increase productivity and, ultimately, profitability.

There are two primary advantages to crossbreeding. First is the ability to combine traits from two or more breeds into one animal. This is called breed complementary. The second advantage is hybrid vigor, also known as heterosis, resulting from crossing animals of different breeds.

Breed complementary results when crossbred animals exhibit desirable characteristics from each parent's breed, resulting in a more valuable animal. This phenomenon allows breeders to blend the superior traits of one animal with the superior traits of another animal into their crossbred offspring. For example, the Zebu Goudali cattle are known for adaptability to hot and humid climates, whereas Italian Simmental and other temperate breeds cattle are known for superior maternal traits resulting from benefits of accrued years of painstaking breed-specific selection schemes. When such crosses are made, their offspring are generally expected to be maternal animals adapted to hot and humid climates. Similarly, Continental breeds would typically inject additional growth performance into a mating with Zebu or temperate breeds. Mating animals of

different breed backgrounds can enhance carcass traits, growth rates, and reproductive performance.

Hybrid vigor, or heterosis, is the increased production of certain traits from the crossing of genetically different individuals. The offspring exceed the average performance of their parents for traits for which hybrid vigor is expressed.

According to MSU (2013), a variety of crossbreeding systems are available for breeders to use in genetic improvement programs. These systems vary in the direct and maternal hybrid vigor they produce, the number of breeding pastures they require, the number of breeds utilized, optimal practical herd size, whether or not replacement females are produced or purchased, labor and management requirements, and timing of herd sire purchases.

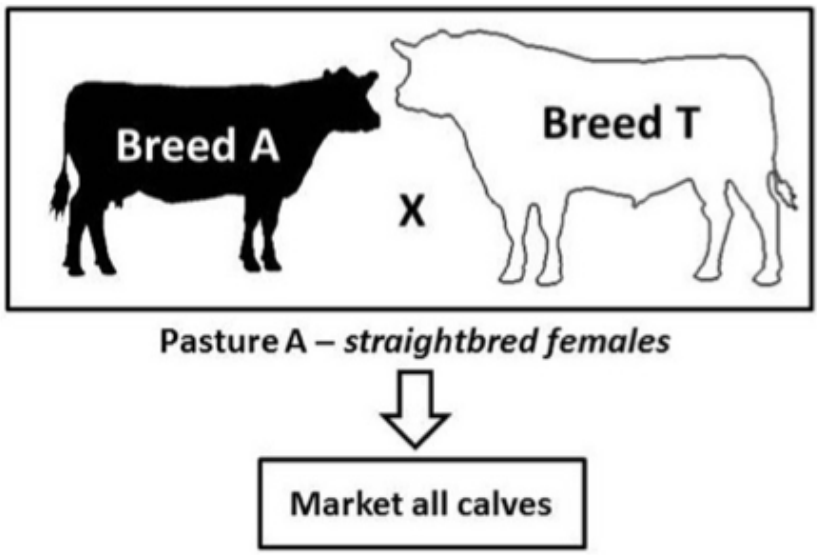

Figure 3: Two-breed terminal crossbreeding system

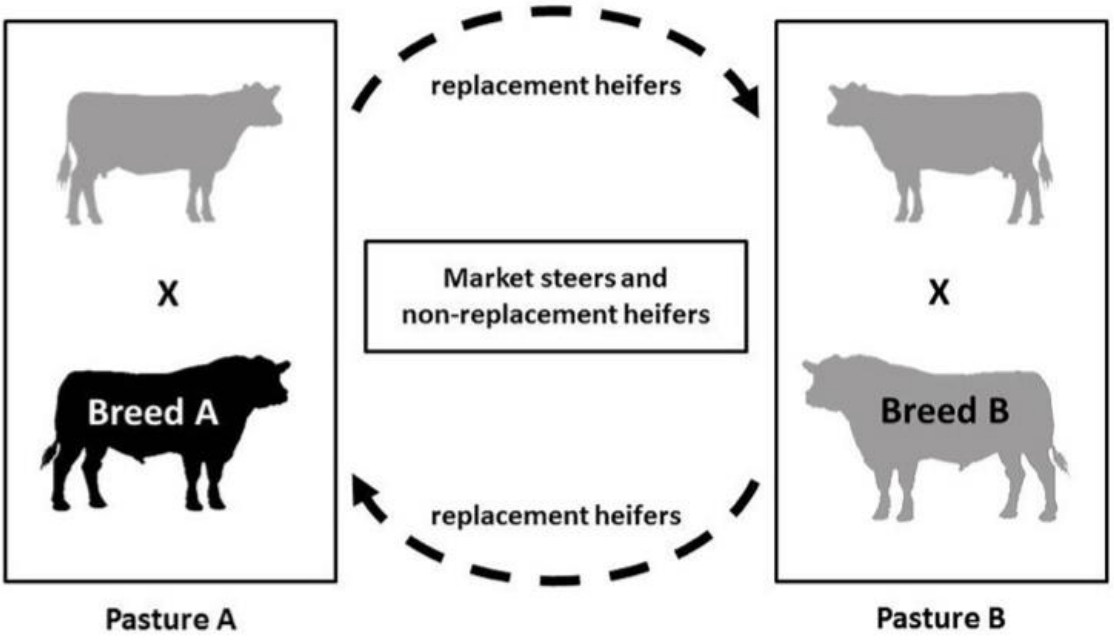

Figure 4: Two-breed rotational crossbreeding scheme

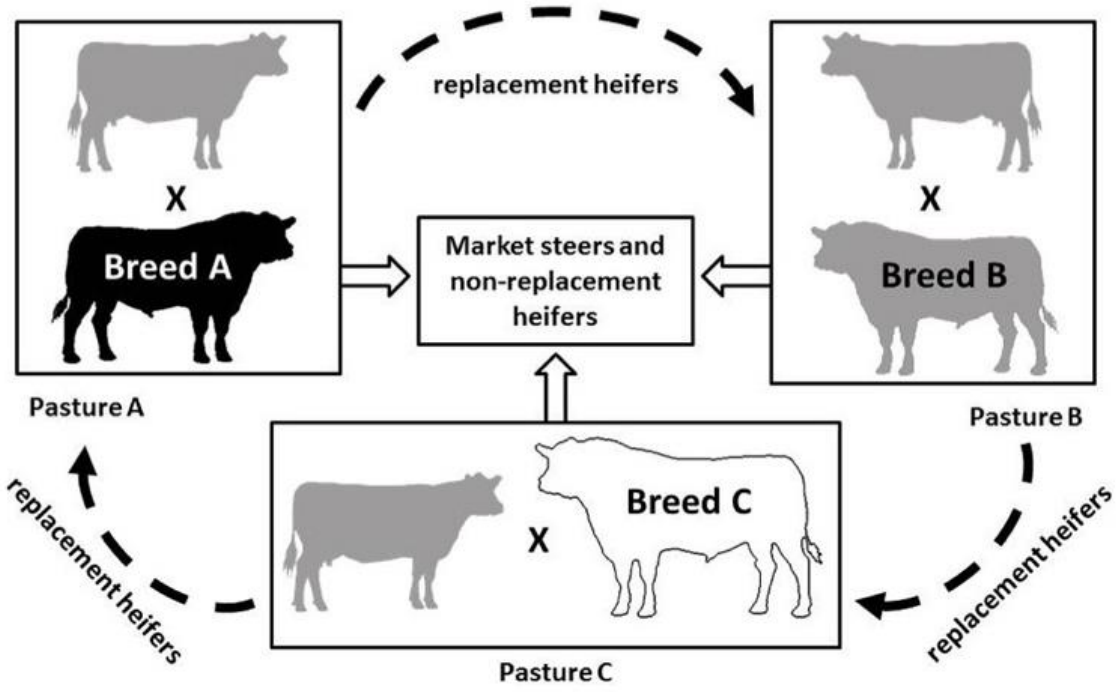

Figure 5: Three breed rotational crossbreeding system

Table 6: Summary of beef cattle crossbreeding systems details and considerations

Breeding Program	Program Details	Considerations	Practical minimum size of cowherd
Two-breed Terminal	• 1 breeding pasture • 1 sire breed • Low labor requirement • Uniform type progeny	• No maternal hybrid vigor • Replacements purchased	Any size
Three-breed Terminal	• 1 breeding pasture • 1 sire breed • Low labor requirement • Uniform type progeny • Maximum hybrid vigor achieved	• Replacements purchased	Any size
Two-breed Rotation	• 2 breeding pastures • 2 sire breeds • Mid-level management • Moderate maternal hybrid vigor • Moderate individual hybrid vigor • Replacement heifers retained	• Maternal/growth sire required • Additional labor • Additional management • Female and sire ID crucial	>50 head
Three-breed Rotation	• 3 breeding pastures • 3 sire breeds • Mid-level management • High maternal hybrid vigor • Higher individual hybrid vigor • Replacement heifers retained	• Maternal/growth sires required • Additional labor • Additional management • Female and sire ID crucial	>75 head
Four-breed Rotation	• 4 breeding pastures • 4 sire breeds • High-level management • High maternal hybrid vigor • High individual hybrid vigor • Replacement heifers retained	• Maternal/growth sires required • Additional management • Female and sire ID crucial	>100 head
Rototerminal	• 3 breeding pastures • 2 sire breed (rotation) + 1 sire breed (terminal) • High-level management • High maternal hybrid vigor • High individual hybrid vigor • Replacement heifers retained	• Maternal sires and terminal sires needed • High-level management • Female and sire ID crucial	>100 head
Sire Rotation	• 1 breeding pasture • 1 sire breed • Low-level management • Low labor requirement • Moderate hybrid vigor • Replacement heifers retained	• Avoids interbreeding	Any size

Source: MSU (2013)

1.7. Current political orientation of cattle husbandry

The Economic Community of Central African States (ECCAS) has CEBEVIRHA as an intraregional political platform created in the 1990s as a regional charter for the promotion and devel- opment of livestock and fisheries. Since the year 2010 ECCAS has engaged in the formulation of a community agricultural policy in conformity with undertakings by heads of african states in Maputo in 2003 to reinforce NEPAD with a detailed agricultural orientation for the development of African Agriculture. Central Africa's livestock sector has the potential to

deliver both the agricultural-led growth, and the socio-economic transformation envisioned in the Malabo Declaration (The Malabo Declaration on Accelerated Agricultural Growth and Transformation for Shared Prosperity and Improved Livelihoods was adopted at the Twenty Third Ordinary Session of the AU Assembly in Malabo, Equatorial Guinea, from 26-27 June 2014, under the Theme of the African Year of Agriculture and Food Security. Under the aegis of this declaration, and from an African continental perspective, the economic subregion is orientating more than ever before towards actions which enhances her pursuit of agricultural-led growth by enhance investment finance, both public and private, to agriculture; in order to end hunger in Africa by 2025, through actions that target on food and nutrition security and shared prosperity.

Central Africa is endowed with enormous land, water and pasture resources, most of which are under-utilized and under-developed. Furthermore, most of livestock production is raised on natural pasture and has the potential to attract niche markets if well promoted. However, the sector faces a various challenges that hinder it from meeting the rising demand for livestock and livestock products and from making a significant contribution to economic growth. Assessments carried out in the five geographical regions of Africa as a whole indicate that the sector is affected by various issues including deficiencies in breeds, production capacities, productivity, availability of quality land, feed and water resources, animal health systems and disease control measures, input supply and service delivery, value addition, market information and market infrastructure, competitiveness of livestock products and in the application of and compliance with sanitary and phytosanitary standards (Rushton *et al*., 2002). These are coupled with deficiencies in policy, legislative and institutional frameworks as well as the inadequate application of available technologies, knowledge and skills.

Owing to the strong attachment to livestock by the pastoral communities in Central Africa, any poverty alleviation goal targeted at pastoral communities will have to focus on strategies to improve livestock productivity by minimising some of the industry's constraints. This is the basic driver of this research study.

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CHAPTER TWO

RESEARCH OBJECTIVES

2 Research objectives

2.1 Overall objectives

Against a backdrop of the above-cited problems plaguing the livestock sector in Central Africa, most especially the near-pristine character of cattle production, this study from a global perspective sought to investigate into baseline strategies that can improve cattle production and productivity in low input farming systems by investigating the case of Goudali Zebu Cattle under Ranching Conditions in Western Highland Sudan-Savannah of Cameroon; taking into consideration key issues on the entire value chain of its welfare and beef production. The study targets the quantitative and qualitative improvement of Goudali cattle productivity through the exploitation of innovative biotechnology and investigations on alternative animal feed proteins source as tools in fostering and facilitating genetic gains and environmental robustness in cattle populations.

2.2 Specific objectives

2.2.1 Body and meat characteristics of young bulls from local Goudali (zebu) and its crosses with the Italian Simmental

Considering the quasi-pristine character of the different cattle production systems prevailing in Central Africa (with the complete absence of conventional animal breeding programmes with a genealogical information system that can engender real-time genetic progress) against the backdrop of a galloping demand for beef and its derivatives mass selection is a logical first step in the development of production. Information on body measurements at the end of growth (in vivo), at slaughter performance and beef characteristics may provide useful insights on the entire beef value chain for different cattle genotypes in the sub-region.

2.2.2 Prevalence of bovine dermatophilosis and disease-associated alleles in Goudali cattle and their Italian Simmental Crosses ranching in the Western Highland plateau savannah of Cameroon

Dermatophilus congolensis is a cosmopolitan and important infectious disease of economic importance to cattle production in the Western highland plateau savannah of Cameroon; Central Africa. The development of naturally resistant animals through marker-assisted selection and breeding is a strong and environmentally friendly alternative to current prevalent control measures. To the best of our knowledge, there is no available information relating to indigenous cattle breeds susceptible to dermatophilosis and studies associating the disease with resistance-marker alleles in Central Africa. This study sought to determine the prevalence of dermatophilosis among Goudali (G) cattle and their Italian Simmental crosses (SG) and to determine for the first time, the distribution of dermatophilosis associated alleles of the Bovine leucocyte antigen DRB3 (BoLA-DRB3) of the major histocompatibility (MHC) locus by allele specific polymerase chain reaction (PCR) among field clinical and non-clinical cases in the Western Highland Plateau Savannah of Cameroon.

2.2.3. Suitability of the use of Jatropha curcas as a novel protein animal feed ingredient in Cameroon

Most of the different production systems described above depend directly and solely on natural pastures for mainly cellulose. Adequate nutrition is vital to expression of the full genetic potential of livestock. Considering the determinant role of ruminant feed in shaping the various production systems and even more importantly the insidious negative impact of dearth of protein feed ingredients to cattle nutrition we studied the suitability of a low-toxic Jatropha curcas cake, extracted from the seeds of a promising biofuel crop, as an alternative protein integrator for ruminant nutrition in a bid to find a possible relief to the pressure on conventional legume cereals such as soybean derived from sources in high competition for arable land and its seeds as direct human food in Central Africa.

CHAPTER THREE

Body and Meat Characteristics of Young Bulls from Auchtotonous zebu Goudali (G) of Cameroon and its crosses with the Italian Simmental (SG)

3.1 Introduction

The cattle population of Cameroon is estimated at 5,805,297 heads (MINEPIA, 2014). Cattle production in Cameroon is essentially conducted by traditional small–scale husbandry management system. Cattle are raised in a wide range of different production and ecological systems and there are variations in genotypes, housing, diet composition and feed availability. However, natural pastures are the major source of feed for cattle. Consequently, major problems are bound, especially in the area of pasture degradation; the feeding problem has become crucial compared to that of animal health, which, thirty years ago, was reported to be the major constraint to livestock farming (Ndi *et al*., 1998). The main cause of pasture degradation is overstocking and poor forage management, which reduce the available supply of pastoral resources. This problem is aggravated by the variations of pastoral resources due principally to climatic fluctuation, which affect the quantity and the quality of natural forage. The resulting effect of these variations is a significant decrease in livestock production performance in the dry season (Nfor *et al*., 2014a; Rippstein, 1985) thereby constituting an important obstacle to the socioeconomic development of the country. This could explain the low per capita meat consumption (13.5 kg/year) as compared to the minimum of 45 kg/year recommended by FAO (2009) (Table 1). The live weight cattle is estimated to range between 150 to 350Kg with a carcass yield of 52%. The rate of exploitation of cattle herds is projected at 40% below their potential weight (EPIA, 2013). Beef appears to be the main source of meat supply (Table 2). The most part of beef is directly produced in the country. There is a non-quantifiable local trans-border trade of live cattle among neighbouring countries, such as Cameroon, Nigeria, Chad and Central Africa Republic. There is a unidirectional movement of live cattle from Cameroon to Gabon and Equatorial Guinea mainly for slaughter.

Table 1. Annual meat consumption in Cameroon (year 2005; FAO, 2009).

Item	Amount
Meat (kg/capita/year)	13.5
Energy (kcal/capita/day)	28
Protein (g/capita/day)	6.9

The Central Africa sub-region is characterized by a low regional trade flow of food products, and EU is its most important commercial partner (Agritrade, 2013). In the years ahead, this region is expected to show an annual growth rate in consumption of beef of 3.2%, however, at the same time; also the imports of beef will increase. Otherwise, Cameroon could also have a strong potential for live animal export to neighbouring countries.

Table 2. Sources of meat supply in Cameroon (year 2005; FAO, 2009).

Reference	Beef	Poultry	Sheep	Pork	Total
Total (thousand tonnes/year)	92	30	32	16	170
Per capita (kg/year)	7.31	2.38	2.54	1.27	13.50

To address the deficit in supply of proteins in Cameroon, there is a need to improve beef productivity not only in quantity but also in quality. Among the factors that affect cattle productivity, the role of the genetic and environmental effects is of greater importance. Genotypes do not perform equally under the same production system and managerial conditions. Various papers have emphasized the existence of genotype by environment interaction for production traits (Boettcher *et al*., 2003; Fikse *et al*., 2003).

In the last 20 years, the transfer of genetic material, started to occur on a very large scale, both within the developed world and from developed to developing countries. This genetic program led to an increase commercialization of the breeding industry, to meet up the rise in demand for animal products in the developing countries. It has equally incited the need to improve new reproductive biotechnologies that can facilitate the movements of genetic material, and the feasibility to control production environments irrespective of the geographical location. Well structured breeding programs provide a key means to increase production levels and product

quality, to increase productivity and cost efficiency, to maintain genetic diversity and to support the conservation and sustainable utilization of specific breeds. Within this approach, it is very important to ensure that breed-genetic diversity is not compromise.

Selection is considered not only as a powerful method to unify desired characteristics, especially in meat animals, but also to stimulate new variations. Selection considers models based on the inclusion of additive genetic effects for the estimations of breeding values, while heterosis requires the use of the dominance model. The interest of crossbreeding is growing among producers to improve some economically important traits characterized by low heritability, such as fertility, health, longevity and calving ease. Crossbred animals may lead to an advantage if economically important traits show heterosis, but the mere evidence of non-additive genetic effects is not enough to state that crossbred are better than individuals purebred. McAllister (2002) reported that the total genetic makeup of crossbred can include additive effects, dominance, maternal effects, maternal heterosis, and recombination effects.

The theory of crossbreeding has been widely reviewed by several authors in the past (Sheridan, 1981; Shoeman *et al*. 1993), and reviews on practical results obtained in beef cattle are available (Long, 1980). Crossbreeding between dairy and beef cattle breeds has also been investigated by several authors in the past (Cundiff, 1970) and, more recently, there has been a major re- search on this subject in the US (Cundiff *et al*., 2005). In a much more recent study, Wolfovà *et al*. (2007) confirmed that carcasses from beef x dairy crosses were much more valuable than carcasses from purebred dairy animals. Moreover, crossbreds showed better eating characteristics of the meat and greater dressing percentage (Güngör *et al*., 2006) than purebreds. Crossbreeding is also important because it leads to a major possibility of the animal to cope with stress due to environmental changes.

Cross breeding beef cattle offers two primary advantages (relative to use of only one breed): the first point is that the crossbred animals exhibit heterosis (hybrid vigor). The second important point is that the crossbred animals combine the strength of the various breeds used to form the cross. Heterosis refers to the superiority in performance of the crossbred animal compared to the average of their parents. However, the goal of a well-designed, systematic crossbreeding program is to, simultaneously, optimize these advantages of heterosis and breed complementarity.

The rotational crossbreeding system is required to exploit breed and heterotic effects. This scheme allows the commercial farmers to produce crossbred female replacements from their own herds. The advantage of rotational crossbreeding is that only purebred sires are required, as crossbred dams are self-replacing (López-Villalobos, 1998). This simplifies the management.

There is a considerable amount of improvement in production from crossing *Bos indicus x Bos taurus* in the tropical countries. It is clear that improvement of indigenous cattle and their utilization play an important role in developing the cattle industry, which can translate into national food security and income growth for cattle producers. Gregory and Cundiff (1980) have demonstrated variously that crossbreeding optimizes the additive genetic and non-additive genetic effects of *B. taurus* and *B. indicus* cattle breeds. Rege *et al*. (1994) and Talbott (1996) also reported similar results. In addition to the additive difference between the two breeds, their first crosses show an improvement in performance due to the fact that they possess 100% heterozygosity with respect the breed of origin (Kahi *et al*., 2000).

In many situations, rotational crossing is preferred to terminal one, because it has the advantage that young females are not slaughtered for meat. It is also important in the circumstance where the aim is to increase the herd. It is based on farmer's experience rather than on genetic theory.

In Africa, the most important crisscrossing program are, for example, Boran x Hereford (in East Africa) and zebu x European beef cross in tropical countries. Tropical breeds are genetically late maturing animals compared to temperate breeds, so adoption of crossbreeding may assist in reducing the animal's age at first calving and improve fertility of the animals.

Different breeding methods are employed to exploit the different kinds of genetic variance in the total genetic improvement of beef production. Selection within indigenous cattle breeds is the only viable production strategy because of the adverse climatic and nutritional condition. The meat production potential is still poorly developed within the indigenous cattle however there is a need to improve the potential to a satisfactory level, without sacrificing adaptation qualities.

The local Goudali breed is widely reared throughout all the various agro-ecologic zone of Cameroon. For this reason, the objective is to improve the Goudali cattle breed with another, exotic breed that can maximize some important characteristics for the production of meat and milk.

These characteristics could be, for example, good growth rate, appreciable tolerance to endemic disease (trypanosomosis and tick-borne disease), ecologically friendly, resistance, and dual purpose. The "Italian Simmental" is a suitable exotic breed for its peculiar characteristics: the milk production that can help also calve rearing, its resistance to diseases, good growth rate, conformation and adaptability. It is important to underline that both these breeds (Goudali and Italian Simmental) are not at risk (or potentially at), so there is not the hazard of losing biodiversity.

The specific objective of the crossbreeding between indigenous or exotic bulls on crossed dams, was to establish and maintain a nucleus herd of dual purpose crossbreed cattle comprising Simmental x Goudali crosses, to allow the exploitation of heterosis from crossbreeding between the zebu and the Italian Simmental and to obtain more productive cattle, adapted to the different production system in Cameroon.

The shape and dimensions of animal carcasses have been variously used to describe carcass fatness (Mohamed, 2004; Alberti *et al*., 2005; Eltahir, 2007), and some carcass measurements such as length, width depth have been recommended as useful predictors of carcass yield and composition. In the context of this study data on carcass characteristics and quality are largely absent and the most widely accepted distinction between beef quality is beef "with bone" or "without bones". The march towards emergence and its associated efforts at developing the beef industry calls for an urgent need for more incisive information on current product characteristics that can predict commercial value as well as provide verifiable guidelines for various beef cattle production objectives.

3.1.1 The Goudali local breed

The Goudali breed constitutes about 60% of the total cattle production in Cameroon and remains the most popular, especially in the smallholder sector of the Adamawa highlands (Bayemi *et al*., 2008; Tawah *et al*., 1996). It is predominantly found in Ngaoundere, in the Adamawa Region of Cameroon, with some strains found in Banyo and Mbere. This local breed is a short

horned West African zebu, predominantly a subtype of the Adamawa Goudali that inhabits the Adamawa mountains ranges stretching from Nigeria to Cameroon (Tawah & Rege 1996). The breed is of good temperament and possesses a natural ability to produce and reproduce optimally under prevailing local conditions without much additional inputs (Tawah *et al*., 1993). The Goudali's head is well proportioned and long, averaging 50-62 cm, and narrow below eyes, averaging 21-25 cm width, with a similar facial profile as in Sokoto Goudali. The hump is very large and pendulous, generally hanging over on one side and having the appearance of being broken. Horn are short to medium in length and crescent-shaped. They are not thick.

3.1.2 The Italian Simmental (IS)

The Italian Simmental is a dual-purpose breed, with many important characteristics useful for improving the local Goudali breed in Cameroon. Thanks to a well-structured and perennial selection scheme, the Italian Simmental has a rapid growth, good beef characteristics and appreciable lactating ability. The average yield per lactation is around 6.5 tons of milk with 3.90% fat and 3.43% protein. This production is excellent, considering that it is obtained with about 45% of cows bred in mountain area. It has an early sexual maturity and docile disposition. It is resistant also to many diseases such as mastitis (Piasentier *et al*., 2010). The coat is red brindle painted (and that, often, can be colourless). The head is white painted (but sometimes there are red spots on it) and the ears are red. The ventral region of the body is white. It has short horns that are yellow waxy coloured, as the hoofs. Its height at withers is about 140 cm and its body weight is between 650-700 kg. at maturity.

3.2. Objective of Study

The objective of this component of the research was to describe and evaluate the SimGoud crossbreed (Simmental x Goudali) first filial (F1) young bull crop of the crossbreeding program carried out by the *Societé de Développement et d'Exploitation des Productions Animales (SODEPA;* Cameroon Livestock Development and Husbandry Corporation) in technical collaboration with the Italian Simmental Breeders' Association (ANAPRI) and the University of Udine (UNIUD). This controlled crossbreeding program targets the establishment of a more productive stock with a relatively improved potential for beef and milk that can diversify and

improve significantly the income of livestock farmers in Cameroon. The aim of the first five years of the crossing program is to create, evaluate and select, groups characterized by a different percentage of Italian Simmental (IS) blood: 75% IS blood (by inseminating the F1 females with IS semen), 50% IS blood (by inseminating F1 females with F1 males from different parents), 25% IS blood (by inseminating the F1 females with Goudali males and Goudali females with F1 males) and 0% IS blood (control pure Goudali group).

We here present and discuss the growth performance and meat yield of F1 young bulls, by comparing body size, growth and composition of Goudali (G) breed with its crosses with Italian Simmental (IS).

3.3. Materials and Methods

3.3.1. Experimental animals

Three hundred and thirty Zebu Goudali (G) cows were recruited to constitute a breeding herd based on the following criteria: be between 5 to 8 years old, with at least two successful parturitions, of good mothering instincts, clinically healthy, nursing a calf of 1-3 months at point of recruitment, and be in good body condition. The selected cows were individually identified by use of plastic ear-tags and corresponding rumen transponders and then cordoned off within 600 ha of the 38,000 ha of Dumbo Ranch, located at Latitude 06° 42' North and Longitude 10° 25' East. They were organized into five artificial insemination breeding herds. Oestrus was synchronized and the cows bred using frozen 0.25 cc straw-type doses of semen from 13 different Italian Simmental bulls (IS). After calving each of the IS x G crossbred calf (SimGoud, SG) was identified by use of a plastic ear-tag and a corresponding rumen transponder. To constitute a control against which the performances of the crossbred calves could be monitored, pure bred calves born about the same week by Goudali cows on a natural mount in the same ranch under the same production environment, were equally identified and subjected to the same nutritional plan (herbage grazed on the Western Highland Plateau Savannah pasture plus NaCl supplementation) and zoo-veterinary care, by introducing them immediately after calving in the artificial insemination breeding herds.

Grazing and management were essentially extensive on natural pastures growing on granitic and basaltic soils. The pastures were composed principally of *Hyparrhenia spp, Panicum maximum, Andropogon guyanensis* and *Pennisetum purpurreum* (Piot & Rippstein, 1975).

Heath management routine involved dipping against ticks, vaccinations against pasteurellosis, brucellosis, anthrax and rinderpest and de-worming. Trypanosomiasis which is one of the most important protozoan blood parasites was controlled by biannual premonition with 1g Isomethamidium Chloride solution at 2% concentration.

Figure 1. Representative sample of Goudali bull at three years age.

Figure 2. Representative sample of SimGoud bull at three years age.

Figure 3. Representative sample of SimGoud bull at four year age.

Figure 4. Representative sample of SimGoud bull at four year age.

Forty-two months after the first SG calving, 50 young male bulls of the genotypes, from 20 to 41 months (Figures 2 and 3), were randomly selected in groups of 10 per experimental herds. The 25 SG young 5 different sired bulls IS bulls while the S ones originated from 5 herds on natural mount of the ranch.

3.3.2. Body Measures

The main body dimensions of bulls were measured before leaving the ranch. In particular, the total top line, neck length, hip height, heart girth, rump length and width (at pinbones), shoulder width and chest depth were taken. The total top line is the total length of the animal (Figure 1; Annex 1), taken from front of the pool to back of the rump. It is taken with three measurements: neck length, body length (equal to the total top line minus the neck length) and rump length (see below). The neck length (Figure 1; Annex 1) is the distance from front of the pool to the middle dip in vertebrate (chine bone) between the shoulder blades.

The hip height is the height of the cattle on the vertical line passing through the hips (Figure 2; Annex 1). The thoracic circumference or heart girth (Figure 3; Annex 1) is the total distance around the animal taken at heart point. The flank girth (Figure 3; Annex 1) is the total distance around the animal taken at the hips. The rump lenght is the distance taken from the hips to the pin bones. In Figure 4 (Annex 1) rump lenght and shape of Goudali and SimGoud bulls are shown. In the Figure 5 (Annex 1) is shown the rump width, that is the horizontal distance between the pin bones. The shoulder width (Figure 6; Annex 1) is the horizontal distance between shoulders, taken from the point of shoulder as the red line underlines. The chest depth (Figure 7; Annex 1) is the distance taken with vertical calliper through the vertical transverse plane passing just to the rear of the point of the elbow. The following biometric indexes were then calculated: mass index (MI = body weight at farm x 100/hip height) and chest depth index (CDI = chest depth x 100/hip height).

3.3.3. Animal transfer and slaughtering procedure

The animals were weighed (body weight at farm, BW farm) and moved initially on-foot for 8 days over 208 km to Bamenda and then loaded in unspecialised animal transport trucks, as is commonly practiced by cattle traders, to Douala in an 8 hours drive over 306 km. To alley stress the animals were rested for five weeks at the Douala Cattle market lairage during which they were grazed intermittently on native pastures on the outskirts of the town close to the market. After this period the animals were slaughtered in the SODEPA industrial abattoir at Douala. The

experimental animals were fasted overnight from feed and then latter-on slaughtered according to the Muslim practice by severing both jugular veins and carotid arteries by a sharp knife without stunning. After complete bleeding the head was removed at the atlanto-occipital joint and weighed. The hide was cut along the limbs and down the abdomen then removed manually and weighed. The fore and hind feet were removed with a knife at the proximal end of the metacarpal and metatarsal joints, respectively, and each was weighed with its hide cover. The tail was separated at the first inter-coccygeal articulation and weighed. After dressing and evisceration, the internal organs and offal were individually weighed. The alimentary tract was weighed and then cleaned of its contents (fill) and reweighed. The weight of fill was subtracted from the slaughter weight to determine the empty body weight (E.B.W.). The kidneys and their surrounding fats were left attached to the carcass. Immediately after slaughter, the fifth quarter (FQ) components and the hot carcass weight (CW) were recorded and used to estimate the individual reconstructed *ante mortem* BW (BWam), the approximate empty body weight (EB = BWam - filled gut) and the transfer losses (Tlosses% = 100 x [BWfarm - BWam]/BWfarm). After chilling for 4ºC for 24 hours, the half carcasses were weighted to obtain the cold carcass weight (CC) and the killing out percentage on the BWam KO%.

3.3.4. Meat Characteristics

After chilling for 24 hours, from the left side of carcass, a sample joint was removed from the seventh to eighth ribs section and dissected in lean, fat, bone and other tissue portion (Andrighetto *et al*., 1996).

The *Longissimus thoracis* muscle from this section (LT7) was sampled and the ultimate pH (pHu) was measured, in three different points, by a pH-meter (HI 8424; Hanna Instruments, Padova, Italy) equipped with a glass electrode (5232; Crison, Barcelona, Spain). The sample was divided in two aliquotes that were separately vacuum-packed, rapidly frozen and stored at -20°C until proximate and fatty acids analysis at the Farming Systems and Product Quality Laboratory of the Department of Agricultural Science and Biotechnology of the University of Udine, Italy.

The proximate composition (AOAC, 2000) was performed on the first aliquot of LT7. The fatty

acid analysis was carried out on the second aliquot of LT7. Extraction of total lipids was performed according to the procedure of Folch *et al*. (1957). A total of 15 mg of nonadecanoic acid (C19:0) were added to a 1.5 g sample of minced meat and homogenized in 30 mL of a chloroform-methanol mixture (2:1 v/v) using an Ultra-Turrax homogenizer (T25 basic; Ika-Werke, Staufen, Germany). The sample was subsequently filtered under vacuum through a Whatman filter paper (No. 1820-047). The extract was washed with 8.5 mL of 0.88% (w/v) KCl, mixed vigorously for 60 s and then left overnight at room temperature. The organic phase was separated, and the solvents were evaporated under vacuum at 40°C. Fatty acid methyl esters (FAME) were prepared using methanolic HCl (Sukhija & Palmquist, 1988). Lipid samples were mixed with 2 mL of hexane and 3 mL of methanolic HCl in 20 mL glass tubes with Teflon lined caps. The mixture was heated at 70°C for 2 hours and then cooled to room temperature. The FAME were extracted in 2 mL of hexane after the addition of 5 mL of 6% (w/v) K_2CO_3 and $Na2SO_4$ anhydrous. Samples stayed for 30 min prior to centrifugation at 1,000 g for 10 min at 20°C. The upper hexane layer was then removed, concentrated under N_2 and diluted in hexane. The FAME were separated using a Carlo Erba gas chromatograph (GC) (HRGC 5300 mega-series; Rodano, Milano, Italy) fitted with an automatic sampler (A200S; Rodano, Milano, Italy) and a flame ionization detector (FID). A 1-µL sample was injected in 1:30 split mode.

The GC was equipped with a 60 m SP-2380 fused silica capillary column (0.25 mm i.d., film thickness 0.25 µm; Supelco Inc., Bellafonte, PA, USA), and the oven temperature was increased from 160 to 180°C at 1°C/min, from 180 to 260°C at 5°C/min and then held at 260°C for 5 min. Helium was used as the carrier gas at the rate of 1.2 mL/min, and FAME were identified using external standards (Supelco 37-component FAME mix including conjugated linoleic acids; Sigma-Aldrich, Milano, Italy). The FAME were quantified using C19:0 as the internal standard and were expressed as the percentage of the total lipids that were identified.

3.3.5. Statistical Analysis

The normality of the data distribution was tested using the Kolmogorov-Smirnov test. The effect of genotype was evaluated by the analysis of covariance using 'genotype' (G *vs.* SG) as a fixed factor and 'age' as a covariate, an intra-class covariate when the intra-genotype coefficients were significantly different. In tables, the genotype means were adjusted to a covariate

mean age of 31 months. The allometry coefficients were calculated by regressing the natural logarithm (ln) of the body component on the ln of the body (EBW or BWam).

3.4. Results and discussion

3.4.1. Body dimensions

The body dimensions of the two genotypes are presented in Table 3, together with the age coefficient of them. This coefficient describes the average month variation of the dimension. It was tabled when significant. The mean values for the two genotypes were adjusted for a reference age of 31 months.

The SG crosses were more sized than the coetaneous pure G. All the body dimensions except two, the chest depth and the neck length, were statistically larger in the crossing bulls that also showed higher age coefficients. At 31 months of age, SG bulls were 9% taller, 11% longer, 24-43% wider than the coetaneous pure autochthonous bulls. The measurements of G bulls appear in line with data reported in literature (Tawah & Rege, 1996). SG bulls increased their size in comparison with pure G bulls without decreasing their adaptability and vigor; indeed the heart girth was equal to total top line in both genotypes. A large girth is needed for proper size for vital organs (heart, lungs, glands), and the closer this measure is with the top line, the more efficient, adaptable and vigorous the animal is (http://www.bovineengineering.com/lin-era_male.html).

The flank circumference is a fertility and maternal trait indicator in females. It was larger than heart girth in both genotypes. Large flank measurement is indicative of presence of meat on rump. The mass index of 31-month old SG males was 48% higher than that of the same aged S, which however showed a low body weight on height ratio in comparison with that reported by Crimella *et al*. (2003). These authors calculated a MI of 2.30 and 2.49 kg/cm respectively for Adamawa Goudali of both sexes weighing 280 and 307 kg, at 24 and 36 months. However, the height at withers corresponding to these values, i.e. 122 and 123 cm for G cattle of 24 and 36 months, are in line with our records.

Table 3. Body dimensions (cm), taken at farm, of young bulls of different genotype (Goudali, G; SimGoud, SG).

Parameter	G	SG	MSE	Age coefficient	
				G	SG
Total topline	145[b]	161[a]	1.01	0.68[**]	1,35[**]
Neck length	49[a]	44[b]	1.36		
Hip height	124[b]	135[a]	0.58	0.41[**]	0.82[**]
Heart girth	148[b]	168[a]	0.87	0.74[**]	1.69[**]
Flank circumference	157[b]	176[a]	0.85	0.59[**]	1.63[**]
Chest depth	58	60	0.93	0.37[(*)]	0.64[**]
Shoulder width	35[b]	50[a]	0.59	0.36[*]	0.67[**]
Rump length	35[b]	40[a]	0.48	0.19[(*)]	0.39[**]
Pinbones width	21[b]	26[a]	0.51	0.23[(*)]	0.41[**]
Mass index (MI)	177[b]	262[a]	2.27	1.55[**]	5.38[**]
Chest depth index (CDI)	46	45	0.70		

[(*)]: P<0.10; [a,b] or [*]: P<0.05; [**]: P<0.01

Chest depth and CDI did not differ between genotype and were slightly lower than expected; indeed, CDI averaged 45-46% instead of 48-49% as reported by Crimella *et al*. (2003). The rump length was larger in SG than G bulls, however the percentage the rump makes up of the body length (100 x rump length/[total topline - neck length]) was similar between genotypes (34.2 *vs.* 36.4%, in SG *vs.* G respectively) and will make quite adequate milking daughters. SG were wider at shoulder than G bulls in both absolute and adjusted value. The last one is calculated by subtracting the rump length from the absolute shoulder width. Wide shoulders makes room for vital organs like heart and lungs.

3.4.2. Body weight, carcass and fifth quarter characteristics

As expected, SG showed higher BWfarm than G (P<0.05; Table 4), with a four times higher growth rate between 20 to 41 months (9.46 *vs.* 2.57 kg/month). This result is due to the combination of additive and heterosis gene effects. In particular, considering that the F1 crosses are considered, the expected breed additive contributor and heterosis effect is 50% and 100% respectively. Demeke *et al*. (2003), crossing Simmental breed with three different *Bos indicus* breeds in tropical Africa improved the yearling weight from 19 to 20%. However, many authors reported that heterosis effect is modulated by environment and production system (Barlow, 1981; Gama *et al*., 2013).

It is interesting to note that SG, despite having higher BWam than G (P<0.05), showed a marked loss of weight during transfer and lairage time (-6%). Conversely, G, during this period was able to increase BW of 4.4%. These results could be due to the higher nutrient requirements and/or to the lower adaptability to transfer condition of the crosses, SG, in comparison with the pure breed, G. Other authors study the metabolic response to load time stress during transportation of cattle (in Nigeria). Severe pre-slaughter stress has been reported to adversely affect meat quality (Apple *et al*., 1995; Warner *et al*., 1986). Plasma glucose concentration have been used as reliable indicator of long time physical stress in cattle (Knet & Ewbank, 1986; Nwe *et al*., 1996; Sanbouri, 1991; Tarrant *et al*., 1992). Stress in cattle due to feed deprivation during transportation have been reported to cause protein breakdown that increases plasma urea N (Kannan *et al*., 2000; Kouakou *et al*., 1999). The authors also noted that as a response to stress, elevation of glucose concentration was preceded by an elevation of cortisol concentration. Therefore, plasma glucose concentration might be useful as an indicator of the intensity of stress (Sanbouri, 1991). These factors have direct negative effect on optimum production and the energy available for maintenance and production (Verstegen, 1987; Von Borell, 2001). They also weaken significantly the body resistance to diseases by depressing cellular and humeral immunity.

Table 4. Weight of body, at farm and ante mortem, fifth quarter and cold carcass; losses during transfer and KO percentage of young bulls of different genotype (Goudali, G; SimGoud, SG).

Parameter	G	SG	MSE	Age coefficient	
				G	SG
BWfarm (kg)	220[b]	354[a]	3.22	2.57[**]	9.46[**]
Tlosses (%)	-4.4[b]	6.1[a]	.763	0.48[**]	0.81[**]
BWam (kg)	228[b]	330[a]	2.39	2.26[**]	5.52[**]
FQ (kg)	104[b]	139[a]	1.27	0.56	0.80[*]
CC (kg)	103[b]	159[a]	1.61	0.89[*]	4.82[**]
KO (%)	45.1[b]	48.1[a]	.347	0.10	0.54[**]

[a,b] or [*]: P<0.05; [**]: P<0.01.
BW farm = body weight at farm; Tlosses = transfer losses; BWam = *ante mortem* body weight; FQ = fifth quarter; CC = cold carcass weight; KO = killing out.

At slaughter, SG showed higher carcass weight and KO% than G (P<0.05; Table 4) confirming the superiority of the F1 crosses in comparison with the pure breed. In a survey performed in Cameroon that involved G bulls from different production systems, Nfor *et al.* (2014a) recorded a hot carcass weight of 152 kg at 4 years. Williams *et al.* (2001) highlighted a positive heterosis effect on carcass weight crossing *Bos taurus* and *Bos indicus* breeds. Considering the fifth quarter composition, SG showed a significantly lower percentage of feet, tail and filled gut, but similar percentage of head, skin and pluck (P>0.05) than G as reported in Table 5. As expected, the allometry coefficient was lower than 1 for head, feet and pluck in both experimental groups indicating that this parts are early maturing respect to EBW (Table 5). Filled gut also showed a diminutive allometry in relationship with BWam. Other authors considered three different breeds such as Afrikaner, Nguni and Pedi and obtained more or less comparable data on head and feet contribution to body weight (Swanepoel, 1990).

Table 5. Fifth quarter composition (% BWam) and allometry coefficients in relationship with EBW (Goudali, G; SimGoud, SG).

	G	SG	MSE	Allometry	
				G	SG
Head	5.75	5.53	0.067	0.85**	0.85**
Feet	3.24a	2.93b	0.069	0.29*	0.31*
Tail	1.18A	0.84B	0.042	0.60	0.57
Skin	3.99	3.76	0.138	1.33**	1.28**
Pluck	3.11	3.36	0.082	0.78**	0.80**
Filled gut[1]	28.03A	25.02B	0.527	0.18	0.22

[1]Allometry coefficient on BWam; A,B or **: P<0.01; a,b or *: P<0.05.

As reported in Table 6, SG showed significantly (P<0.05) higher weight of rib steak and ribeye muscle at 8th-9th rib section level than G (P<0.05). The covariate, age, was significantly related to weight of rib steak and ribeye muscle for SG (21.5 and 5.9 g/month respectively, P<0.01), but not for G (1.7 and 0.7 respectively, P>0.05; data not reported in tables). These results indicate that SG had a greater growth than G. In particular, considering the sampling joint composition, SG had higher percentage of lean tissue and similar percentage of fat and bone tissue than G (P<0.05; Table 6).

Table 6. Weight and tissue composition of the sampling cut (8th-9th rib section) from young bulls of different genotype (Goudali, G; SimGoud, SG).

	G	SG	MSE
Rib steak weight (g)	510b	760a	17.2
Ribeye muscle (g)	102b	173a	4.96
Lean (%)	66.3b	68.9a	0.358
Fat (%)	3.9	2.8	0.363
Bone (%)	24.6	24.3	0.765

a,b: P<0.05.

Corazzin *et al*. (2012) in a study that considered Simmental young bulls fed with concentrate, reported a sampling joint composition of 64.7% meat, 14.0% fat and 17.0% bone. Perotto *et al.* (2000) crossing Nellore, a *Bos indicus* breed, with Simmental, observed an increase, despite not significant, of 2.7% of the percentage of lean meat of sample joint at 12^{th} rib. Theunissen *et al*. (2014), crossing *Bos taurus* and *Bos indicus* breeds observed an heterosis effect of +0.8% on meat yield that was estimated considering the dissection of sample joint at 8^{th}-10^{th} rib level. Considering the sample joint and KO results, it could be speculated that SG had better carcass conformation at slaughter than G.

3.4.3. Meat composition

LT composition is shown in Table 7. Differences between G and SG were not found (P>0.05). Marshall (1994) reviewing the effects of different breed crosses reported an average positive heterosis effect of 3.8% for marbling. Conversely, Gama *et al*. (2013), crossing *Bos taurus* and *Bos indicus* breeds in pasture finishing conditions, showed a significant heterosis effect for moisture, +1.4%, but not for fat, protein and ash.

Table 7. Percentage chemical composition of *Longissimus thoracis* muscles (g/100 g fresh meat) of young bulls of different genotype (Goudali, G; SimGoud, SG).

	G	SG	MSE
Moisture	76.6	76.0	0.227
Protein	20.1	20.5	0.248
Fat	0.60	0.76	0.053
Ash	1.06	1.06	0.017

The above-cited authors explained that heterosis effect is strongly influenced by the animals' diet. Consequently, in our study, the lack of additive and heterosis effects at slaughter on meat fat content could be due to the severe transfer conditions of bulls from farm to slaughterhouse that have caused a probable reduction of the final fat level in muscle. Indeed, the average fat level was low, 0.68%, much lower than the value reported by Nfor *et al*. (2014b), 1.34% in G reared in Cameroon and with similar feeding conditions, but transported to slaughterhouse by

truck. Savell and Cross (1988) suggested a minimum of 3% fat to ensure an acceptable palatability of beef. The average protein level found, 20.3%, fell within the range proposed for beef by Muchenje *et al*. (2009) of 20.0-22.9%, but it was lower than those showed by Salifou *et al*. (2013) in zebu Fulani, 21.7%, and by Nfor *et al*. (2014a) in G bulls, 22.1%, both reared on natural pasture in tropical environment. The total lipid weight and relative proportion of FA in muscle *Longissimus thoracis* according to genotype and age are shown in Table 8. The intramuscular fat content of meat was low and did not differ between genotypes, even if the SG beef tended to be fatter than G one.

Table 8. Total lipids (g/100 g fresh meat) content and fatty acid profile (% of total lipids) of *Longissimus thoracis* muscle of young bulls of different genotype (Goudali, G; SimGoud, SG).

	G	SG	MSE	Age coefficient
Lipids tot (g/100 g meat)	0.71^{β}	0.95^{α}	0.065	
C8:0	0.28	0.27	0.033	
C10:0	0.40	0.46	0.034	
C11:0	0.02	0.03	0.004	
C12:0	0.17	0.17	0.013	
C13:0	0.03	0.04	0.011	
C14:0	1.77^{α}	1.41^{β}	0.094	
C14:1	0.40^{α}	0.34^{β}	0.017	
C15:0	0.83	0.39	0.149	
C15:1	0.29	0.27	0.014	
C16:0	21.44	21.03	0.305	
C16:1cis7	0.69	0.77	0.029	
C16:1n-9	0.75	0.68	0.032	
C17:0	1.36	1.33	0.039	
C17:1	0.55	0.53	0.028	
C18:0	26.39	24.75	0.537	$0.165^{(*)}$
^{1}C18 :1t	2.40	2.39	0.135	
C18:1n-9	22.72^{B}	24.76^{A}	0.303	

(continue)

	G	SG	MSE	Age coefficient
C18:1n-7	1.13^{B}	1.40^{A}	0.030	
C18:2n-6	7.60	8.44	0.445	
C18:3n-6	0.32^{α}	0.28^{β}	0.010	
C18:3n-3	1.76	1.99	0.087	
CLAt7, c9/t8, c10/c9, t11	0.23^{a}	0.16^{b}	0.015	
C20:3n-6	0.16	0.15	0.018	-0.015^{**}
C22:0	0.41	0.39	0.045	
C20:3n-3	0.31	0.28	0.046	-0.018^{*}
C20:4n-6	3.44	3.15	0.256	-0.110^{*}
C22:1n-9	0.06	0.09	0.015	$-0.005^{(*)}$
C23:0	0.16	0.17	0.015	
C22:2	0.22	0.20	0.025	
C20:5n-3	1.34	1.42	0.103	-0.043^{*}
C24:0	0.18	0.17	0.020	
C24:1n-9	0.16	0.15	0.014	$-0.004^{(*)}$
C22:5n-3	1.77	1.82	0.104	-0.042^{*}
C22:6n-3	0.23	0.12	0.041	
SFA	53.43	50.61	0.767	$0.231^{(*)}$
MUFA	29.08^{B}	31.31^{A}	0.386	
PUFA	17.49	18.08	0.973	$-0.321^{(*)}$
Total odd FA	3.24	2.76	0.163	
PUFAn6	11.76	12.18	0.684	$-0.216^{(*)}$
PUFAn3	5.42	5.62	0.300	$-0.100^{(*)}$
n6:n3	2.15	2.18	0.035	

[1]C18 :1t corresponds to the summ of : t6-8-, t9-, t10-, t11-, t12--, t13/14- 18:1.

$^{\alpha,\beta}$ or $^{(*)}$: P<0.10; ^{a,b} or *: P<0.05; ^{A,B} or **: P<0.01.

Overall, genotype had a limited effect on FA profile in the current study. This is in agreement with the general conclusion of the (De Smet *et al*., 2004). In studies with Nguni and Bonsmara cattle (Muchenje *et al.*, 2009), breed was also reported to have a small effect on FA composition of meat. However, the FA profile of G beef tended to have a greater content of meristic, meristotelic and C18:3n-6 acids and showed a greater CLA level than SG beef. While, SG had higher MUFA content, and particularly C18:1n-9 and C18:1n-7. The CLA percentage of the two genotypes, 0.16 and 0.23 respectively for SG and G, falls within the range reported in literature for zebu and zebu derived cattle (0.15 to 0.43 % of the total lipids) raised on natural pastures around the world (De Mendoza *et al*., 2005; Muchenje *et al*., 2009; Salifou *et al*., 2013). In the current study, n-6/n-3 PUFA ratio was similar across breeds and n-6/n-3 PUFA ratio was 2.16, a value comparable to that observed in zebu of Cameroon by Nfor *et al*. (2014b). From a human health perspective, the maximum recommended n-6/n-3 PUFA ratio to reduce the risk of coronary heart disease is 4.0 (English Department of Health, 1994). The natural pasture-based diet, characterized by a high proportion of linolenic acid (Webb & Erasmus, 2013), may explain the lack of differences between genotypes and the low ratio of n-6/n-3 of beef. With the age increase in both genotypes there was an increase of SFA, stearic in particular, that replaced long-chained PUFA.

3.5. Conclusions

The results of this study showed that G pure breed has much lower *in vivo* and slaughter performance than their crosses with Simmental breed. This is probably because of the additive and heterosis effects that interact with severe conditions of the animal. This result highlights the possibility, in order to maximize the crossbreeding effects, to improve bull's transfer and pre-slaughter conditions.

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CHAPTER FOUR

Prevalence of bovine dermatophilosis and disease associated alleles in zebu Goudali cattle and their Italian Simmental crosses ranching in the western highland plateau savannah of Cameroon

4.1 Abstract

Abundance of native pastures makes Cameroon's western highland savannah (WHS) a hotspot for low-input beef-type cattle. Dumbo Ranch is central to cattle seed-stock multiplication in WHS and holds that *Dermatophilus congolensis infection* undermines production. The bovine BoLA-DRB3 has been variously demonstrated as the principal gene of the major histocompatibility locus associated with immunity and resistance to dermatophilosis in cattle. We studied the profile of dermatophilosis prevalence in zebu Goudali (G) and its Simmental composite SimGoud (SG) at Dumbo Ranch and determined the distribution of dermatophilosis-associated alleles of the BoLA-DRB3 gene by allele-specific Polymerase Chain Reaction (PCR). We recorded a 42 % prevalence of dermatophilosis in the studied cohort (total number of animals=337). Dermatophilosis tended to be more common in females (χ^2=2.892; p=0.088) and in cattle ≤ to 36 months than in older cattle (χ^2=4.259; p=0.039). SG was more affected compared to G (χ^2=9.169; p=0.002). No susceptible homozygote was observed. Eighty-five and fifteen percent of the cohort carried the homozygous resistant and heterozygous condition respectively. Differences in the distribution of alleles among zebu cattle types was not significant (χ^2=0.046; p=0.829). The study confirms the presence of dermatophilosis among G and SG cattle in WHS. However, there was no correlation between the presence of disease-associated susceptible alleles and clinical manifestation. Monogenic screening for diseases-associated alleles of BoLA-DRB3 gene appeared not to be useful to select G and SG for dermatophilosis resistance in WHS.

Keywords: Goudali; SimGoud; Dermatophilosis; Genetic resistance; Cameroon.

4.2 Introduction

Dermatophilosis is an economically important disease of livestock and also an agent of zoonotic importance, caused by an actinomycete *Dermatophilus congolensis* (Albrecht *et al*., 1974; Burd *et al*., 2007; Gillespie & Timoney, 1981; Pal, 1995). In cattle, it presents clinically as an exu-

dative skin disease complicated by *Amblyoma variegatum*, which is known to be intricately associated with its epidemiology predisposing affected animals to skin lesions (Ambrose *et al*., 1999). The disease is reported most frequently in relatively low altitude areas with tropical and subtropical climates. Factors such as prolonged wetting by rain, high humidity, high temperature, mechanical injury of skin, concurrent disease, stress and tick infestation increase prevalence, seasonal incidence and transmission of dermatophilosis.

The disease was first reported in the Belgian Congo (Van Sacegham, 1915), it is now known to be cosmopolitan (Zaria, 1993). Loss in reproduction in cows and heifers is affected by debilitation and loss in body condition caused by the disease, and calves of dams with infected udders starving to death. In breeding bulls, debility and ulcerations on the scrotal skin and leg extremities water down libido and semen characteristics (Sekoni, 1993). Cost of therapy and chemoprophylaxis, cost in time and effort at the control of disease, as well as loss resulting from high culling rates impact negatively on the industry. Loss in productivity has been reported to be up to 15% (Lloyd, 1976). Morrow *et al*. (1993) reported the disease as one of the main constraints in improving the productivity of cattle in West and Central Africa.

Dermatophilosis can be managed by rigorous tick control and treatment of clinical cases. However, in the western highland savannah (WHS) of Cameroon where cattle rearing is preponderantly pastoral and transhumant, recommended control measures have not been successful. Irregularity in supply and high cost of acaricides, insufficiencies in appropriate acaricide application materials and dipping infrastructure, development of chemoresistance to acaricide and antibiotics pose enormous challenges to cattle production and development.
Research on vaccines for prevention of dermatophilosis has been conducted (How *et al*., 1990, Sutherland & Robertson, 1988), but no vaccine is currently available (OIE, 2008). Much emphasis has therefore been put on tick control and identification of genetic markers of resistance or susceptibility with promising results in cattle (Maillard *et al*., 2003).

Despite severe dermatophilosis morbidity and mortality on some animals, there is an observed consensus among breeders and cattle farmers that not all breeds and subjects of the same herd are affected clinically to the same extent even when under the same care. The situation ranges

from clinically unaffected to mild, through severe chronic infections to death, in animals in all age groups.

The BoLA-DRB3 has been variously shown to be the principal gene of the major histocompatibility (MCH) locus associated with hypersensitivity, controlling the immune response and resistance to *Dermatophilus congolensis* in some cattle breeds (Maillard *et al*., 1999; Razafindraibe *et al*., 2006). The antigen presentation site (APS) is encoded by exon 2 of the DRB3 gene. The BoLA-DRB3*09 allele shows a particular "C-E-S-F-L-QK-N" aminoacid sequence in the APS positions 11-28-30-37-67-70/71-74, which is a motif marker of susceptibility. In a previous study, elimination of animals possessing this haplotype by marker-assisted selection reduced the incidence of dermatophilosis from 76% to 2% over a period of 5 years (Maillard *et al*., 2002).

Goudali (G) zebu cattle has been indicated to have a moderate resistance to dermatophilosis (Mattioli *et al*., 1995; Koney *et al*., 1994). The development of naturally resistant animals through marker-assisted selection and breeding is a strong and environmental friendly alternative to the current control methods. To the best of our knowledge, there is no available information relating to indigenous cattle breeds susceptible to dermatophilosis and studies associating the disease with resistance-marker alleles in Cameroon and the west and central African sub-region.

This study sought to determine the prevalence of dermatophilosis among G cattle and their Simmental crosses [SimGoud (SG)], and to determine, for the first time, the distribution of dermatophilosis-associated alleles of the BoLA-DRB3 gene of the MHC locus by allele specific Polymerase Chain reaction (PCR) among field clinical and non-clinical cases in WHS of Cameroon.

4.3 MATERIALS AND METHODS

4.3.1 Study location

SODEPA Dumbo is a 38,000 ha ranch, located in the WHS of the Northwest Region of Cameroon, latitude 06° 42' North and longitude 010° 25' East, in a tsetse fly belt with *Trypanosoma vivax* prevalence (Figure 1). The climate is subtropical, characterized by a bimodal rainfall pattern. Annual precipitation ranges from 20,600 mm to 26,436 mm, while relative humidity ranges from highs of 95 % to lows of 50 %. The area, like most parts of the WHS, is infested with a wide variety of ticks, notably *Amblyoma spp.*, and other insect vectors such as tsetse flies, and midges, which favour the endemicity of trypanosomosis, cowdriosis and ephemeral fever (Esemu *et al.;* 2013)

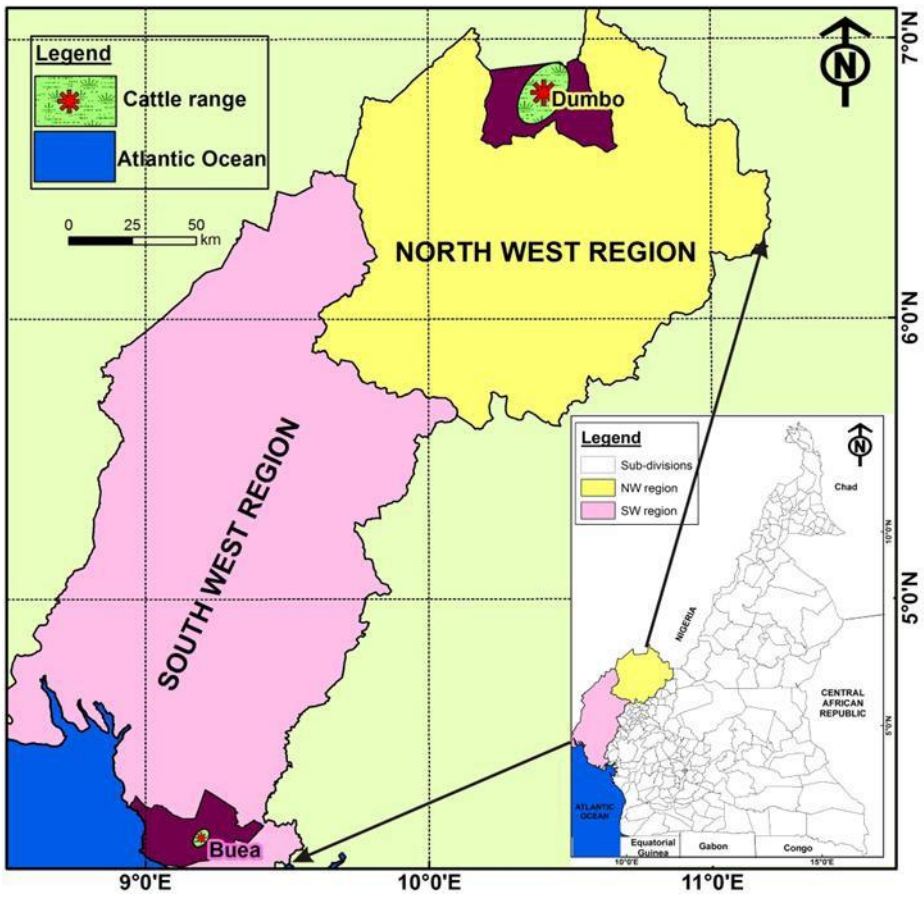

Figure 1. Geographical location of SODEPA Dumbo Cattle Ranch in the Northwest Region of Cameroon.

4.3.2 Experimental Animals

Animals were sampled from two different cohorts of cattle, comprising cows, bulls, calves, heifers and young bulls, reared in the same pastures and well identified by a serial numbering on plastic eartags (Allflex®). The first group (SG) comprised animals produced by artificial inseminating of *Bos indicus* G cows with *Bos taurus* Italian Simmental (IS) frozen semen, and a second group comprising straight breed G cattle produced by natural mount, from herds in the same ranch under the same production environment, in an improved breeding program that started in 2008 (Bessong *et al*., 2015). On the whole, 337 cattle of G and SG cattle types were controlled, of which 211 were females. Birth records from the herds, in combination with physical examination provided information on the breed, age, and sex of the animals

The animals were grazed exclusively on native ranch pastures, consisting preponderantly of *Hyperhnaria spp.* and to a lesser extent S*porobolus spp.* and *Pennisetum spp.* Generally, good quality pasture encountered during the rainy season was truncated by the harsh dry season characterized by poor pastures with dismal nutrient value. The only supplement administered to the animals was common salt (NaCl), administered bi-weekly.

Health monitoring was carried out on a regular basis. Bacterial, viral, protozoal infections, tick-borne diseases such as dermatophilosis are endemic in the stock population, as well as cattle in the adjacent communities. Cattle were vaccinated against contagious bovine pleuropneumonia, black quarter, nodular dermatitis, and pasteurellosis annually. One gram Isomethamedium chloride was administered bi-annually on the herds, in October and April, corresponding to the beginning and the end of the dry season, as premonition against tsetse fly transmitted trypanosomosis. To control external parasites, tick burden and mitigate tick borne infections cattle were dipped in 2% acaricide (cypermethrin solution).

4.3.3 Assessment of clinical dermatophilosis

Dermatophilosis was classified as: mild, if lesions were confined to just a few raised hair with underlying papules confined to the neck, face and dewlap regions without matting; moderate, if the lesions, in addition to the situation of mild, extended to the back with matting of hair on affected areas; severe, if the lesions were generalized matting and encrusted involving wide areas of the skin, extremities, udder, testicles and were life-threatening with a need for culling. Animals without clinical lesions related to dermatophilosis were considered unaffected.

4.3.4 Sample collection and total DNA extraction

Five millilitres of whole blood was collected from each animal for genetic determination of dermatophilosis allele-associated mutations in the BoLA-DRB3 gene. At the Laboratory for Emerging Infectious Diseases of the University of Buea; Cameroon, DNA was purified with the Qiagen Mini Blood DNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Purified DNA was stored at -20°C until used.

4.3.5 Detection of dermatophilosis associated mutations by allele-specific PCR

Alleles previously associated for homozygous resistance, heterozygous and homozygous susceptible to dermatophilosis (Maillard *et al*., 2003) were determined by allele specific PCR amplification. The primers used were Bod31 (5'- GATGGATCCTCTCTCTGCAG-CACATTTCCT-3'), Bod32 (5'-CTTGAATTCGCGCTCACCTCGCCGCTG-3') and an inner primer PASA (5'- GCGGGCGGGGTTCCTGGAGAGATC-3'), as previously described by Maillard *et al*. (1996; 2003). The amplification reactions were performed in a final volume of 20 µl, which contained Bod 31 and Bod 32 primers (6 pmoles each), PASA primer (4 pmoles), 10 ul iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, Unites States), and 50 ng of DNA. The amplification was carried out in a Perkin Elmer 2400 thermal cycler (Perkin Elmer, Waltham, MA, Unites States) under the following conditions: initial denaturation at 95°C for 3 minutes; followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 59°C for 30 seconds, elongation at 72°C for 30 seconds, and final extension at 72°C for 5 minutes.
As a result of PCR, a 307 bp product represents any BoLA-DRB3 resistant allele, a 235 bp is expected for homozygous susceptible allele, while a 307 bp together with a 235 bp is expected for a heterozygous condition. Amplification products were resolved by 2% agarose gel electrophoresis and viewed under UV translumination.

4.3.6 Statistical Analysis

The association between the allele condition and cattle type, age, gender and clinical manifestation of dermatophilosis was analyzed by cross-tabulation and Chi square test.

4.4 Results

Despite acaricide treatment, cases of dermatophilosis lesions occur among cattle herds. Table 1 shows the distribution of clinical dermatophilosis with respect to gender, age and animal type. Overall, dermatophilosis was clinically detected in 143 of the 337 cattle (42.4%). Of the clini- cally detected presentations 96 (67.1%), 28 (19.6%) and 19 (13.3%) animals had mild, moder- ate and severe dermatophilosis respectively. Dermatophilosis was more common in females than in males, but this was not statistically significant (χ^2=2.892; p=0.088). Interestingly, dis- ease burden was slightly more significantly common in cattle less than or equal to 36 months of age than in older cattle (χ^2=4.259; p=0.039), already in reproduction in the ranch production system. From a cattle type perspective, SG cattle were the most affected (95/337, 28.2%) com- pared to G (48/337, 14.2%), and this observation was statistically significant (χ^2=9.169; p=0.002).

Table 1. Percentage distribution of grade of clinical dermatophilosis with respect to gender, age and cattle type (total number of animals=337).

Trait		Absent	Mild	Moderate	Severe	χ^2	p
Gender	Male	80	32	3	11	2.892	0.088
	Female	114	64	25	8		
Age, months	≤ 36	185	89	24	15	4.359	0.039
	≥ 37	9	7	4	4		
Cattle type[a]	G	37	22	22	4	9.169	0.002
	SG	157	74	6	15		
Total (%)		194 (57.5)	96 (28.5)	28 (8.3)	19 (5.7)		

[a] G=Goudali; SG=SimGoud.

Table 2 shows the distribution of BoLA-DRB3 genotypes according to gender, age and animal type. No homozygous susceptible genotype was observed in the study cohort. However, 14.8% (50/337) of the cohort possessed the heterozygous condition, while 85.2% (287/337) harbored the homozygous resistant allele condition.

Table 2. Distribution of BoLA-DRB3 genotypes according to gender, age and cattle type (total number of animals=337).

		Homozygous resistant	Heterozygous	Homozygous susceptible	χ^2	*P*
Gender	Male	104	22	0	1.0962	0.295
	Female	183	28	0		
Age	≤ 36	266	47	0	0.1117	0.738
	≥ 37	21	3	0		
Cattle type [a]	G	24	11	0	0.0460	0.82
	SG	213	39	0		
Total (%)		287 (85.2%)	50 (14.8%)	0		

[a] G=Goudali; SG=SimGoud.

Figure 2 shows a representative gel electrophoresis photo of the allele-specific amplification products of the BoLA-DRB3 gene. The difference in the distribution of genotypes between gender was not significant (χ^2=1.0962, p=0.295). The same was the case for the age, between animals less than or equal to 36 months of age and older cattle, already in reproduction (χ^2=0.1117, p=0.738). Also the difference in the distribution of genotypes between G and SG cattle was not significant (χ^2=0.046; p=0.829). Of the 50 hetero- zygous cases, 39 (78%) where in SG cattle, with a frequency of the susceptible allele equal to 8%, and 11 (22%) in the autochthonous zebu that harbored a 6.5% of the susceptible allele.

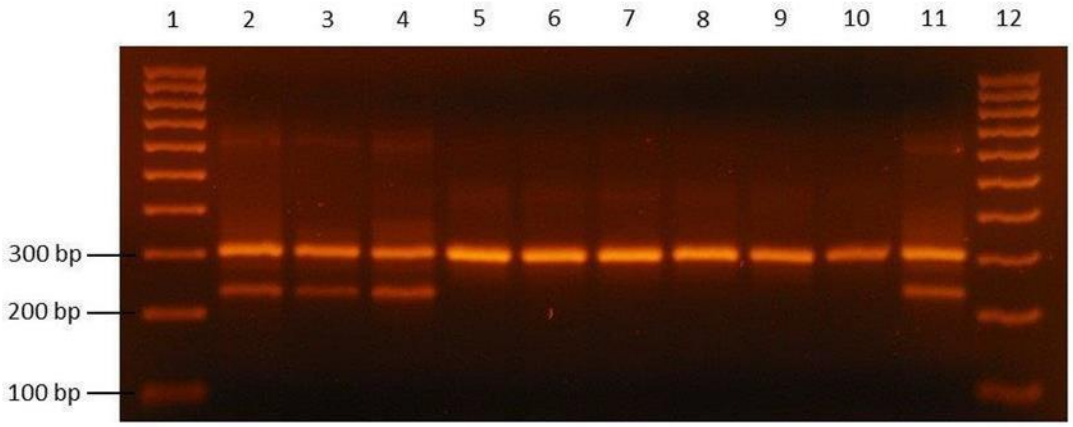

Figure 2. A representative gel photo of amplified alleles of BoLA-DBR3 gene. Lanes 1 and 12: 100 bp molecular marker. Lanes 2 – 11: samples. Lanes 2 – 4 and 11: heterozygote ani- mals (307 bp and 235 bp bands). Lanes 5 – 10: homozygote resistant animals (307 bp band).

Table 3 shows the distribution of BoLA-DRB3 genotypes according to clinical manifestation of dermatophilosis. Twenty-six (52%) of the animals presenting the heterozygous condition did not show any clinical signs of dermatophilosis, while 18 (36%) showed the mild form, and 5 (10%) showed the moderate form of the disease. One young bull carrying the heterozygous condition showed the severe form of the disease. About 58% of animals of the homozygous resistant genotype did not present dermatophilosis symptoms, while about 30% of the homozy- gote animals showed the mild, 8% the moderate and 6% the severe form of the disease.

4.5 Discussion

Dermatophilosis is a common pathology among cattle worldwide with significant implications for animal health. Field assessments have shown different pictures of dermatophilosis. For ex- ample, high susceptibility has been observed among the Friesian breed, while the zebu Sanga breeds of Ghana and the Goudali of Cameroon have intermediate susceptibility to dermatophi- losis, and the West African N'dama display high resistance to of the disease (Ibeagha-Awemu *et al*., 2008). The current study was undertaken to provide baseline data on the prevalence of dermatophilosis in G and SG cattle types in western highland savannah pastures of Sub-Saharan Africa.

Table 3. Distribution of BoLA-DRB3 genotypes according to clinical manifestation of dermatophilosis in the study cohort (total number of animals=337).

Dermatophilosis grade	Genotypes distribution		
	Homozygous resistant	Heterozygous	Homozygous susceptible
Absent	166 (57.8 %)	26 (52 %)	0
Mild	80 (27.9 %)	18 (36 %)	0
Moderate	23 (8 %)	5 (10 %)	0
Severe	18 (6.3 %)	1 (2 %)	0

Despite all control measures, there was still a prevalence of ticks in the herds because of their endemic diffusion in these pastures. Overall, a clinical dermatophilosis incidence of about 42% was observed in the study cohort, with about 67% of these presenting the mild form of the disease. The observed prevalence is twice that of what was reported in a tropical highland region of Ethiopia by Woldemeskel and Taye (2002), and about three times higher than a recent study from Zimbabwe, where a prevalence of about 15% was reported (Ndhlovu & Masika 2015).

In the present study, it was noted that the frequency of dermatophilosis was not significantly associated with gender, in agreement with the study of Kassaye *et al*. (2003). However, a slightly significantly higher prevalence was observed in cattle above 36 months of age. This prevalence estimate with age may probably either be attributed to a possible age dependency or age relatedness because of both survival and disease incidence.

As expected, G breed showed a lower susceptibility to dermatophilosis than SG crossbreed, because its better resistance to the pathogen evolved over years of adaptation in tropical cli- mates. Regarding susceptibility to ticks, many tropical zebu breeds are known to have greater tolerance to tick infestations than taurine breeds of temperate regions or origin (Ibeagha-Awemu *et al*., 2008).

Several studies have been conducted on the association between BoLA polymorphism and dis- ease resistance in cattle, such as paratuberculosis (Restislav & Mangesh 2012), *Boophilus microplus* ticks (Acosta-Rodriquez *et al*., 2005), *Neospora caninum* (Schwab *et al*., 2009) and bovine dermatophilosis

(Maillard *et al*., 2003). The most widely cited example in cattle is re- sistance to mastitis (Chu *et al*., 2012; Dietz *et al*., 1997; Kelm *et al*., 1997; Rupp *et al*., 2007; Schwab *et al*., 2009), which were mainly caused by *Escherichia coli, Klebsiella spp*. and *Strep- tococcus uberis*. Among BoLA genes, the DRB3 is highly expressed and 103 different alleles have been identified, implying its high polymorphism (Hameed *et al*., 2006; Takeshima *et al*., 2002;). Maillard *et al*. (2002) found that a unique BoLA class II haplotype, made up of one DRB3 allele highly correlates with dermatophilosis susceptibility. In our study we analyzed, by using the suggested primers (Maillard *et al*., 1996, 2003), the character region encoded by exon 2 of the DRB3, and in particular the 'C-E-S-F-L-QK-N' amino acid sequence in the APS po- sitions 11-28-30-37-67-70/71-74, which was considered a motif marker of susceptibility. How- ever, we did not find significant association between BoLA-DRB3 genotypes and dermatophi- losis clinical manifestation. The question then to answer is whether screening for these alleles can be used to select breeds resistant to dermatophilosis in pure zebu Goudali and its crosses. It has been reported that genetic profiles do not always translate to phenotypic outcomes, par- ticularly when the protein expression pathway has other variables that play a role in the pheno- type, and also depends on whether the disease is under monogenic or polygenic control (Ibeagha-Awemu *et al*., 2008). Whether it is related to the high prevalence of resistant alleles in the population can only be confirmed with a prospective study that takes into consideration the genetic profile of the major histocompatibility complex gene, and environmental risk factors (Ndhlovu & Masika 2015).

In conclusion, the current study has shown that clinical dermatophilosis is common among zebu G and SG cattle in the western highland plateau savannah of Cameroon and that resistance cannot be predicted solely on the variability of BoLA-DRB3 gene.

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CHAPTER FIVE

USE OF *JATROPHA CURCAS* AS FEED INGREDIENT

5.1 Introduction

The large-scale cultivation of plant based feedstock for bio-energy production has become a reality in a short time. The bio-energy sector is predicted to expand in future. This expansion is triggered mainly by increased petroleum prices, climatic change and energy security concerns. The bio-energy produced from sustainably managed systems could provide a renewable and carbon neutral source of energy. The production of bio-fuels is an attractive solution for mitigating green house gas emissions, enhancing energy security and providing socio-economic benefits. The leading contributors of biomass for bio-fuel production are agriculture, forestry and the wood processing industries. However, continuous feedstock supplies for biofuel production is intimately linked with issues such as competition with food crops, ecotoxicity, and water and land use (Ruane *et al*., 2010).

In a relatively short time, *Jatropha curcas* L., a first generation bio-fuel plant has gained a lot of momentum for the production of biodiesel on a commercial scale. Among the many tropical and subtropical countries involved in large-scale production of Jatropha, India, China and Indonesia are at the forefront. The total worldwide cultivation is expected to increase to 12.8 million hectares by 2015. The current global seed production and projected future seed production is 23 and 43 mMT (GEXSI, 2008). The Jatropha seed oil can be processed into high quality biodiesel, which meets European and American biodiesel standards (Makkar *et al*., 2009a).

Description of the plant

Jatropha is a genus of flowering plants in the spurge family, *Euphorbiaceae*. The name is derived from the Greek words ἰατρός (iatros), meaning "physician", and τροφή (trophe), meaning "nutrition" or “food”, hence the common name physic nut. The genus *Jatropha* is extremely

old and approximately contains 175 species (Dehgan & Webster, 1979) of succulent plants, shrubs and trees (some are deciduous, like *Jatropha curcas* L.). Most of these are native to the Americas, with 66 species found in the Old World. Mature plants produce separate male and female flowers. As with many members of the family *Euphorbiaceae*, *Jatropha sp*. contains compounds that are toxic.

Dehgan and Webster (1979) distinguished two subgenera (*Curcas* and *Jatropha*), ten sections and ten subsections. The subgenus *Curcas* comprises all Mexican, one Costa Rican, two African and one Indian species, while the subgenus *Jatropha* includes all South American, African (except two), Antillean, all Indian (except one) and two North American species (Xu *et al.*, 2012). Most species in this genus are native to Central and South America, the diversity centre of Jatropha (Heller, 1996). Several *Jatropha* species are cultivated for their ornamental leaves and flowers, while some are grown in the tropics for their economic uses. Since some species of this genus having significant economic importance are regarded as potential biodiesel plants, the genus has created tremendous interest all over the world for the biodiesel use (Xu *et al.*, 2012).

Jatropha curcas L. (the most widespread species around the world) is a diploid species with 2n = 22 chromosomes and it is considered as the most primitive species in the genus (Dehgan & Webster, 1979). The Caatingas in Northeast Brazil and the dry areas of Mexico have been identified as the centres of diversity (Makkar & Becker, 2009). From tropical America, *J. curcas* was probably distributed to Africa and Asia by Portuguese seafarers via Cape Verde Islands and Guinea Bissau and, nowadays, it has a pantropical distribution (Achten *et al.*, 2010; Xu *et al*., 2012).

Jatropha curcas L. is shrub or a small- to medium-sized tree distributed in the subtropical and tropical regions of the world. It is stress tolerant, drought resistant, grows in semi-arid, marginal lands, as well as does not compete with conventional food or feed crops for land and water, which makes it as an ideal choice to make use of vast presently underutilized land resources (Heller, 1996). In tropical countries such as India and China, it is well-known as an oilseed, a live hedge and used for prevention of soil erosion (Ye *et al*., 2009).

The *Jatropha curcas* L. has characteristically five lobed leaves, one main taproot and four shal-

low lateral roots. The plant can reach a height of 3 m (Contran *et al.*, 2013), but under favorable conditions it can grow even up to 8–10 m (Divakara *et al.*, 2010) and a life expectancy of up to 50 years (Achten *et al.*, 2008). *J. curcas* shows articulated growth, with a morphological discontinuity at each internode, and its dormancy is induced by rainfall and temperature fluctuations (Heller, 1996). The plant presents terminal and axillary small buds and branches from the ground. Stem is straight, with thin pale-brown bark and numerous scars due to the fallen leaves. Branches are glabrous, stout, and contain latex (Heller, 1996; Kumar & Sharma, 2008). *J. curcas* leaves are green to pale-green, 5–7 lobed, smooth, alternate to sub-opposite with a spiral phyllotaxis, and hypoamphistomatic, with length and width of 6–15 cm, petiole of 5–20 cm long, and paracytic (brachyparacytic) stomata (Abdulrahaman & Oladele, 2011). In climates characterized by a dry season, it is a deciduous plant and sheds its leaves during the dry season (Kumar & Sharma, 2008).

J. curcas initially develops a deep taproot and four main secondary order roots. These four roots, symmetrically distributed in the horizontal plane, originate at the same depth along the main root and have an inclination of −45°. Adult trees present four clearly dominant secondary order roots, with an inclination of −20° and −40°, and develop a more branched root system, with a dense net of finer horizontal lateral root in the topsoil (Reubens *et al.*, 2011). The tap root may stabilize the soil against landslides while the shallow roots are alleged to prevent and control soil erosion caused by wind or water, but this potential has not been investigated scientifically (Achten *et al.*, 2008).

It is monoecious with terminal inflorescences containing unisexual male and female greenish yellowed flowers on the same inflorescence (raceme). Normally, the inflorescences produce a terminal individual female flower surrounded by a group of male flowers. Numerically, 1–5 female flowers and 25–93 male flowers are produced per inflorescence (Raju & Ezradanam, 2002). The higher number of male flowers than female flowers results in a very low yield. The ratio of male to female flowers decreases with the age of the plant and it changes drastically (108:1) with the fall in temperature (Divakara *et al.*, 2010). The inflorescences contain occasionally hermaphroditic flowers (Abdelgadir *et al.*, 2009; Dehgan & Webster, 1979; Raju & Ezradanam, 2002).

The flowering season and the number of flowering events, as well as the male and female flower

ratio, are dependent upon temperature, available soil moisture, and soil fertility (Kant & Wu, 2011). Normally, flowering occurs during the wet season (one or two flowering peaks), but in permanently humid regions *J. curcas* flowers throughout the year (Achten *et al.*, 2008).
Insects pollinate the plant, especially by honey bees (Abdelgadir *et al.*, 2009; Divakara *et al.*, 2010). Jatropha is self-compatible (Abdelgadir *et al.*, 2009; Heller, 1996), but cross pol- lination is supported by a time gap between anthesis of male and female flowers. The existence of protandry may have a strong impact for cultivation of Jatropha hybrids, and its magnitude has to be investigated in more detail (Makkar & Becker, 2009). Negussie *et al.* (2013) reported that *J. curcas* is not only of protandrous nature as reported earlier: in their study, they have repeatedly observed that also female flowers opened first. Therefore, *J. curcas* can be of both protandrous and protogynous nature. Inadequate pollination may limit *J. curcas* seed production and thus oil yield (Carels, 2009).

After pollination, the inflorescence forms a bunch of ovoid green fruit (Carels, 2009; Kochhar *et al.*, 2008). Fruits are green-brown ovoid capsules, 2.0–3.5 g weight, 4 cm long, 3 cm diameter, and generally tri-halved, each comprised of one seed (Heller, 1996). The pericarp remains fleshy, indehiscent, and green until the seeds are mature.

After 2-3 months from fruit formation, when fruits are completely developed and reach maturity, the exocarp dries, forming the husk (sometimes named fruit coat or fruit shell) of the fruit, and its color changes from green to yellow, brown, and finally black (Kumar & Sharma, 2008). The husk opens partly, but seeds do not fall out. In a mature fruit, husk is the 30–40% of the total fruit weight and seeds are the remaining 60–70%. The fruits do not mature all at the same moment (Achten *et al.*, 2008).

Seeds, up to 3 (1-3) per capsule, are black, ellipsoid, triangular-convex, 0.5–0.7 g weight, about 2 cm long and 1 cm thick. The 30–40% of the total seed weight consists of an external brown-black shell (sometimes named hull or seed husk), whereas the remaining 60–70% consists of kernel, the white oil-containing compact nucleolus of seed (Achten *et al.*, 2008). *J. curcas* seed contains about 30–35% of oil per dry mass, stored at 99% in the kernel. The seeds of *J. curcas* contain also a wide range of constituents toxic to humans and animals (Kumar & Sharma,

2008).

Phorbol esters have been identified as the main agent responsible for *J. curcas* toxicity (Makkar *et al.*, 1997). There are two genotypes of *Jatropha curcas,* a toxic and a non-toxic (characterized by free or low phorbol ester seeds) one. The latter genotype is found in Mexico only (Basha *et al*., 2009; Makkar & Becker, 2009). The toxic and harmless varieties cannot be distinguished visually. The seed yield reported for Jatropha varies from 0.5 to 12 tons/ (year ha^{-1}), depending on soil, nutrient and rainfall conditions, genetics, plantage, management (propagation method, spacing, pruning, fertilizing, irrigation, etc.) and the tree has a productive life of over 30 years (Achten *et al.*, 2008; Francis *et al.*, 2005; Heller, 1996; Openshaw, 2000).

The harvested mature fruits are generally sundried (<8% moisture) and the dried fruits is 30−35% husk and 60−65% seeds. The seeds have a heat of combustion of 4980 cal/g (20.85 MJ/kg) with an oil content of 35%. The energy content of the oil is 9036 cal/g (37.83 MJ/kg). Jatropha has wide climatic adaptability and grows where there is rainfall between 250−3000 mm (Foidl *et al.*, 1996) and the temperature between 15−40°C and also survives on marginal lands under harsh climatic conditions. Its cultivation requires simple technology, and comparatively modest capital investment.

Jatropha is classified as the climax vegetation of tropical savannas in the dry or semi-dry tropics. Its drought tolerance and adaptation capacity to long, severely dry seasons are well developed, to a degree that established plants have been reported to grow even where there is no rain for 2-3 years. On the other hand, Jatropha appears to tolerate humid conditions equally well, showing good growth with high rainfall. Jatropha is therefore highly adaptable to varying precipitation conditions. Heavy rains at the time of flowering could lead to the complete loss of flowers. Jatropha does not tolerate instantaneous flooding (Makkar & Becker, 2009).

A temperature range of 25–35°C is optimum for *J. curcas* growth, but in some regions in the tropics it may be found at higher altitudes with the risk of light frost. On the other hand, Jatropha can tolerate elevated temperatures to far above 40°C, as has been documented in a 150-ha plantation in Upper Egypt, Luxor, where on 260 days of the year the temperatures exceed 40°C. Temperatures below 20°C for a week or even shorter periods initiate leaf shedding (Makkar & Becker, 2009) Suitable conditions were found with annual precipitation above 600–900 mm,

with an optimum at 1500 mm (Trabucco *et al.*, 2010). *J. curcas* requires mean annual temperatures between 18°C and 28°C (with optimal values around 26–27°C), average minimum temperatures above 8–9°C, indicating a clear lack of tolerance to frost, and average maximum temperatures between 35°C and 45°C (Trabucco *et al.*, 2010). The plant is not sensitive to day length (Achten *et al.*, 2008).

J. curcas is able to grow in a wide range of soil types, ranging from alluvial soil to red lateritic soil, even on gravelly, sandy, and saline soils (Ye *et al.*, 2009). Neutral (pH 6.0–8.0), well-drained, and aerated soils are preferred, whereas soils with risk of ephemeral water logging, such as Vertisols or other heavy clay soils, are not suitable (Achten *et al.*, 2008). In Nicaragua, it has an altitude range from sea level up to 1800 m (Foidl *et al.*, 1996).
The plant can be easily propagated by either seeds, seedlings or cuttings and has a productive life of 30–50 years (Heller, 1996). Plants propagated vegetatively do not usually form tap roots. The advantage of generative propagation compared to vegetatively propagated plants needs to be substantiated. Seed propagation – even in the case of transplanting nursery-raised seedlings – is clearly less costly but produces a highly variable stand, whilst vegetative propagation (cuttings) allows establishing uniform stands of selected, high-yielding material (Makkar & Becker, 2009)

Product description

It should also be noted that most of the studies have overstated the impacts of first generation biofuels on land and agricultural markets by ignoring the role of biofuel by-products and coproducts. Among the many by-products some, such as oilseed meal, could be utilized in the livestock industry as a source of protein, and coproducts such as bioactive phytochemicals could be utilized in agropharmaceutical applications (Devappa *et al*., 2008; Makkar *et al*., 2009b). The maximum utilization of value added by-products/coproducts could increase the sustaina- bility of the Jatropha biodiesel industry while in turn reducing the impact on land and green house gas emissions by avoiding the disposal or burning of wastes obtained during biodiesel production. The ability to use *J. curcas* meal as animal feed not only improves the economics of *J. curcas* production, but also means the crop would produce both fuel and feed. During the biodiesel production, many coproducts such as seed cake, phytochemicals, and glycerol, among

others could be generated (Makkar & Becker, 2009).

Seed cake (a by-product generated from oil extraction) contains high levels of protein (56-63%), higher than Soybean meals (40-45%). The levels of essential amino acids, except lysine, are higher than in the FAO reference protein for growing children (Makkar *et al.*, 2008). Although it contains a high amount of protein, it has phorbol esters and anti-nutritional factors such as phytate, trypsin inhibitor, lectin and saponin that cannot be applied directly in the food or animal feed industries. Considering the large quantity of seed cake generated after oil extraction and all the possible applications, its commercial use is vital for economic viability of the *J. curcas* system.

Processing of seeds to extract oil

The *Jatropha* species that is most widely studied in a nutritional context is *J. curcas*. The seed contains kernel and shell with an average ratio of 62.2:37.7. The kernel has higher crude protein (22-28%) and oil contents (54-58%) compared to the shell (4-6% crude protein and 0.8-1.4% oil). The de-husked seeds from the ripe fruits should be cleaned, separated from moulds/contaminants and dried before storage in a ventilated room, which is protected against pests. The dehusking and decortication of *Jatropha* seeds are important to give high yields of good quality oil and to reduce the bulk of material to be processed. However, there is no universal standardized procedure for decortications and oil extraction. Generally, Jatropha seeds are pressed manually or with a hydraulic press (screw press) to extract oil. In mechanical pressing such as expeller pressing, heated oilseeds are passed via the feed inlet into one end of a barrel or tube and are conveyed by a rotating worm assembly to the discharge end. This extraction method results in oil yield up to 30−35%, considering the average oil content of Jatropha seeds ranging from 35−40% (Karaj & Muller, 2009). However, when large quantities of oilseed cake have to be pro- cessed, solvent extraction becomes a commercially viable option to extract the residual oil left in the cake and to obtain an almost oil-free powder known as oilseed meal. Generally, solvent extraction plants use hexane as a solvent to extract oil from oilseed cake. These plants are expensive and only suitable for large volumes of material, which justify the capital cost of the equipment. The residual cake and meal obtained after oil extraction are potentially useful by-products. Overall, out of 27-40% oil available in the seed, 60-80% and 70-99% could be recov-

ered by mechanical (screw press) and solvent extraction respectively (Achten *et al*., 2008). The high variability in oil yield and on the nutritional value in the oilseed meals may be attributed to the difference in genotypes of Jatropha seeds and extraction methods used.

Jatropha meals have interesting nutritional and biochemical properties. The protein composition of Jatropha seed kernel meal has been shown to compare favourably with soybean meal, containing a good balance of essential amino acids with the exception of lysine. The ability to use Jatropha meal as animal feed not only improves the economics of Jatropha production, but it also indicates that the crop would produce both fuel and feed. In nutritional context, they are at least comparable to if not better than soybean proteins. Due to its high protein content, high protein digestibility and good amino acid composition make Jatropha proteins a promising supplemental source in the diets of ruminant and monogastric animals including fish. The proximate compositions of meals (solvent extracted meal, SEM, screw-pressed meal, SPM and soybean meal, SM) are shown in Table 1, indicating that SEM and SPM contained a very good nutrient profile, comparable to SM.

Table 1. The proximate compositions of *Jatropha curcas* meals.

	Samples		
	SEM	SPM	Soybean meal (SM) (Vasconcelos et al. 1997)
Crude protein	58.6 ± 0.8^{a}	45.3 ± 1.2^{b}	45.7
Lipid	2.5 ± 0.1^{b}	16.1 ± 0.3^{a}	1.8
Crude ash	10.3 ± 0.2^{a}	10.4 ± 0.5^{a}	6.4
Crude fiber	5.00 ± 0.6^{a}	5.5 ± 0.3^{a}	5.01
Neutral detergent fiber	9.1 ± 0.2^{a}	9.2 ± 0.4^{a}	17.2
Acid detergent fiber	7.9 ± 0.1^{a}	7.8 ± 0.6^{a}	12.2
Gross energy	19.0 ± 0.8^{b}	48.0 ± 1.5^{a}	19.4

All values are means±standard deviation ($n=3$). Values followed by different letters are significant difference ($p<0.05$)

SEM soxhelt extracted meal; *SPM* screw-pressed meal; *SM* soybean meal

Table 2 showed the content of the buffer-soluble nitrogen, non-protein nitrogen, pepsin insoluble nitrogen and in vitro protein digestibility (IVPD) in defatted meals. Only 4.3 - 9.5% of the total nitrogen in SPM and SEM was non-protein nitrogen, suggesting the presence of a high level 90% of true protein. The values of IVPD in the SEM and SPM were 79.7% and 72.8% respectively, lower than those reported in soybean (80.6%), probably due to the high content of TIA, lectin and phytate present in the *Jatropha curcas* meals and the protein had been denatured.

Table 2. In vitro protein digestibility and nitrogen composition of *Jatropha curcas* defatted meal.

	Samples		
	SEM	SPM	Non-toxic meal (Makkar and Becker 2009)
In vitro protein digestibility	79.7 ± 0.3^{a}	72.8 ± 1.2^{b}	80.6
Pepsin insoluble nitrogen	4.2 ± 0.1^{b}	9.1 ± 0^{a}	3.5
Buffer-soluble nitrogen	7.2 ± 0.6^{a}	6.0 ± 0.7^{b}	7.9
No-protein nitrogen	4.3 ± 0^{b}	9.5 ± 1.1^{a}	5.0

All values are means±standard deviation (n=3). Values followed by different letters are significant difference (p<0.05)

SEM soxhelt extracted meal; *SPM* screw-pressed meal

The amino acid composition in SPM, SEM and SM is shown in Table 3. In general, glutamic acid, arginine, aspartic acid and leucine were all abundant, similar to conventional oilseed proteins. In addition, a comparison between the amino acid composition of SPM, SEM and SM revealed an almost similar pattern for all essential amino acids, except lysine and sulphur-amino acids. The levels of essential amino acids in SEM, except lysine, cysteine and phenylalanine, were higher than that of the FAO/WHO reference protein for a five-year-old child on a dry matter basis.

Table 3. The amino acid composition of *Jatropha curcas* meal.

Amino acid	Samples			
	SEM	SPM	SM (Vasconcelos et al. 1997)	FAO/WHO (ref.protein) [a]
Essential				
Cystine	1.01	0.57	1.64	2.50[b]
Methionine	1.11	0.82	1.39	
Valine	4.98	5.61	4.72	3.50
Isoleucine	4.45	3.72	3.98	2.80
Leucine	6.97	8.94	7.61	6.60
Arginine	11.34	10.66	7.47	
Phenylalanine	4.52	5.10	5.76	6.30[c]
Histidine	2.42	2.38	3.03	1.90
Lysine	3.48	2.99	6.84	5.80
threonine	3.62	2.71	3.85	3.40
Tyrosine	2.57	2.80	4.94	
Non-essential				
Aspartic acid	8.75	9.38	11.9	
Proline	5.94	5.75	5.10	
Serine	4.73	5.19	4.15	
Glutamic acid	16.07	11.39	18.6	
Glycine	4.42	5.01	3.93	
Alanine	4.76	6.16	4.19	

SEM soxhelt extracted meal; *SPM* screw-pressed meal; *SM* soybean meal

[a] Reference pattern suggested for pro-school children (2–5 years old)

[b] Methionine plus cystine

[c] Phenylalanine plus Tyrosine

Nutritional indices of the SPM, SEM and SM were given in Table 4. Evaluation of the nutritional value of protein must be based on the content and composition of amino acids, in particular the content and composition ratio of essential amino acids, which is an important factor to determine the nutritional value of protein. The values of nutritional indices of SEM were higher than that of SPM, but lower that of SM. Data also showed that different defatting ways can affect the nutritional quality of protein.

Table 4. Nutritional indices of *Jatropha curcas* meal.

	Samples		
	SEM	SPM	SM (Vasconcelos et al. 1997)
Essential amino acid index	72.4	68.0	80.87
Biological value	67.2	62.4	76.45
Nutritional index	42.5	30.8	34.96
Protein digestibility corrected amino acid score	0.55	0.51	0.68

SEM soxhelt extracted meal; *SPM* screw-pressed meal; *SM* soybean meal

Conclusions regarding the product description

A comparison between the amino acid composition of Jatropha meal and soybean revealed an almost identical pattern for all essential amino acids, except for lysine and the sulphur amino acids; lysine is lower and the sulphur amino acids are higher in the Jatropha meals. The levels of essential amino acids in the Jatropha meals are higher than or similar to those of castor bean meal. The non-protein nitrogen in Jatropha meal formed only 9.0% of the total nitrogen in the Jatropha meals, suggesting the presence of high levels (91%) of true protein.

Digestibility and metabolisable energy of heat-treated (121°C, 66% moisture, 30 min) kernel meal, using the *in vitro* gas method were lower compared to those for soybean meal by 10% units and by 2.5 MJ/kg dry matter. The digestibility of the Jatropha kernel meal protein, determined by treatment with pepsin followed by trypsin, was similar to that of toasted soybean

meal, whereas the *in vitro* rumen digestibility of nitrogen was lower by approx. 50%, suggesting that Jatropha kernel meal has a high level of rumen undegradable protein, which might be available post-ruminally.

These results imply that Jatropha kernel meal could be an excellent protein source for animals having the potential to give high milk, meat and wool yields, once the problem of the presence of antinutritional compounds could be overcome. The solution to the problem could be the use of accessions of *J. curcas that do* not bear these anti-nutritional substances or expected for the Jatropha kernel meal from the toxic genotype once it has been detoxified

Background information on non-toxic varieties of J. curcas

The edible variety of *J. curcas* has been identified and reports available till now mention their origin as Mexico. Its seeds have same or similar chemical composition as the toxic varieties except for the fact that it lacks phorbol esters (Martínez- Herrera *et al*., 2006; He *et al*., 2011). The seeds of the non-toxic genotype are traditionally used in Veracruz, Puebla and Hidalgo States of México for preparing a variety of traditional dishes and local people consume the seed kernels after roasting (Martínez- Herrera *et al*., 2006). Studies have clearly shown that the non-toxic nature is a dominant maternal characteristic and accidental outcrossing with toxic *J. curcas* does not affect the phorbol esters content of seeds borne on non-toxic Jatropha plants (Sujatha *et al*., 2005). The same study showed that plants grown from seeds of non-toxic plants also produced non-toxic seeds. The recent observation of much higher levels of phorbol esters in the maternal tegument of toxic seeds provides further evidence that these terpenoids may be synthesized in the maternal tissues (He *et al*., 2011), further underlining the maternal determi- nation of the presence or absence of phorbol esters in Jatropha plants.

The genetic distinctness of the edible varieties compared to the toxic varieties has been confirmed by molecular marker analysis of Jatropha germplasm (Basha *et al*., 2009; Pamidiamarri *et al*., 2008, He *et al*. (2011) postulated that the genetic existence of two distinct classes of seed based on phorbol esters content, one high and the other at least 1000 times lower, strongly suggests that phorbol esters content is controlled by a single genetic locus.

Chemical and nutritional quality of the edible Jatropha kernels

The chemical composition of the seeds and seed kernel meal and the nutritional quality of edible *J. curcas* has been reported and compared with toxic provenances (Martínez-Herrera *et al*., 2006; 2010). According to the published data, the oil content in seeds and the fatty acid composition of the oil are similar in the edible and non-edible varieties of Jatropha. The amino acid composition was not different for the kernel meal of the toxic and non-toxic varieties and levels of essential amino acids, except lysine, were higher than that of the FAO/WHO reference protein and comparable to that of soybean meal (Martínez-Herrera *et al*., 2006). The composition of antinutrients presented in Table 6, shows clearly that differences between edible and non-edible varieties occur only with regard to the presence or absence of phorbol esters.

Table 5.

Proximate composition, total soluble sugars and starch content of full-fat and defatted seed kernel meal of toxic and non-toxic *J. curcas kernel* meals.

	Crude protein (g/16 gN)	Crude lipid (%)	Crude ash (%)	Crude fibre (%)	Gross energy (kJ/g)	NDF (%)	Total soluble sugars (%)	Starch (%)
Full fat kernel meal								
Non-toxic	34.5	57.2	3.8	2.8	31.5	3.9	4.4	5.9
Toxic	33.6	56.3	3.9	3.4	31.5	4.5	4.4	5.7
Defatted kernel meal								
Non-toxic	64.9	0.4	10.4	4.9	18.2	9.2	10.3	11.2
Toxic	61.9	0.6	10.4	6.1	18.8	10.3	10.2	10.6

Extracted from Martínez-Herrera et al. (2006).

Table 6.

Comparison of antinutrient factors between defatted non-toxic and toxic jatropha kernel meals.

	TI (mg/g sample)[a]	Phytic acid (%)	Total phenolics (g/100 g)[b]	Saponins (g/100 g)[c]	Lectin activity (mg/ml)[d]	Total phorbol esters (mg/g)[e]
Non-toxic	35.95	8.76	0.24	2.14	1.46	ND[f]
Toxic	34.04	8.55	0.18	2.85	1.41	3.85

Extracted from Martínez-Herrera et al. (2006).

[a] TI, mg of pure trypsin inhibited/g sample.
[b] Tannic acid equivalent.
[c] Diosgenin equivalent.
[d] Minimum amount of the sample required to show the agglutination after twofold dilution in 1 ml of final assay medium.
[e] Equivalent to phorbol 12-myristate,13-acetate.
[f] Not detected.

The high nutritional quality of heat treated non-toxic Jatropha kernel meal was proved in several feeding experiments with fish (Richter *et al*., 2005; Richter, 2012) and rats (Martínez-Herrera *et al*., 2012), proving that non-toxic Jatropha kernel meal is a high quality animal feed ingredient.

Comparative evaluation of toxic and nontoxic Jatropha genotypes

Variation in seed number of fruits

The literature reports that the fruits of *J. curcas* contain three seeds. However, fruits with one, two and four seeds were also observed. Within a genotype, the highest percentage of fruits contained three seeds, followed by two seeds for both of the genotypes. The fruits containing three and four seeds have been found to be more frequent for the non-toxic genotype and also the variation was higher for the non-toxic genotype. A higher percentage of fruits with three and four seeds will render a higher yield. On average, 100 fruits of the non-toxic and toxic genotypes would yield 270 and 252 seeds, respectively.

Physical parameters

The seed, shell and kernel masses and the shell/seed ratio (the kernel-to-seed ratio) have been observed to be similar, for both the genotypes. On the other hand, the seed and shell masses of the toxic genotype were higher than those of the nontoxic genotype; however, the kernel masses of the toxic and non-toxic genotypes were statistically similar, suggesting that the higher seed mass of the toxic genotype is contributed by its higher shell mass. From the same number of seeds from the toxic and non-toxic genotypes, the yield of kernels will be the same; however, from the same weight, the yield of shells will be higher from the toxic genotype. The kernel mass as percentage of the seed mass did not differ significantly between the two genotypes (61.5% for the toxic and 63.9% for the non-toxic genotype). Similar values have been observed for seeds obtained from India, Nicaragua, Cape Verde and Mexico.

Oil and protein contents

The oil and protein contents of seeds and kernels have been found to be similar for the toxic and non-toxic genotypes. The above results suggest that 1 t of toxic and non-toxic seeds would yield 615 and 639 kg of kernels, with almost identical oil contents of approximately 57.1 and 56.7%, respectively. The potential oil recovery from 1 t would be 351 and 362 kg for the toxic and non-toxic genotypes using solvent extraction. In other words, to obtain 1 t of oil, 2.85 t of toxic and 2.76 t of non-toxic seeds would be required. When the yield is expressed per unit of seed number (*e.g.* 1000 seeds) the yield of kernels and oil is expected to be similar for the two genotypes. However, from the same weight or same number of seeds, the yield of shells (a good source of energy; see below) will be higher for the toxic genotype.

It may be concluded from these findings that the yield of oil from the seeds of the non-toxic genotype is not inferior to that of the toxic genotype, which is widely used throughout the world. There is a need to evaluate the seed yield per hectare from both the toxic and non-toxic genotypes in different climatic conditions.

Field performance of non-toxic Jatropha plants

At present, it is believed that the non-toxic genotype, since it lacks a kind of toxin (phorbol esters, which are plant defence compounds), would be more prone to various environmental vagaries including pests and diseases. With proper care and management practices, as for any other edible oil seed crop, the nontoxic genotype could give edible oil and seed cake for use in the diets of farm animals and aquaculture species.

Common reservations regarding non-toxic Jatropha plants are:

- lack of good quality non-toxic seeds;
- the fear that they may be more susceptible to pests and diseases because of the absence of phorbol esters;
- fragmentary reports of lower seed yield compared to the common toxic varieties.

Phenotypic growth pattern

The vegetative appearance of both toxic and non-toxic Jatropha plants were more or less similar, except for a slight difference in the nature of the leaves and generally the smaller size of the non-toxic plants compared to the toxic plants.

Pest incidence

Several pests were found to infect the Jatropha plants. The major pests that have been consistently noticed every year included leaf webber/top shoot borer (*Pempelia morosalis*), mealy bugs (*Paracoccus sp.*), Scuttellerid bugs (*Scutellera nobilis*) and red mites (*Tetranychus urticae*). Mealy bug seriously affected the plants and repeated insecticide sprays were necessary to control them that year. The incidence of all other pests was more or less seasonal, did not appear to cause severe damage to plants and could be easily controlled by common pesticides. There were some differences between accessions regarding susceptibility to different pests, but no differences could be ascertained between toxic and non-toxic accessions as far as pest infestations are concerned. All infestations noticed occurred in plants of both toxic and non-toxic accessions.

Chemical composition of Jatropha kernel meal

The contents of crude protein, ash, gross energy and neutral detergent fibre of kernel meal (residue left after solvent extraction of oil from kernels, with kernels being the inner white material left after removal of the shells) are similar for the two genotypes. Sugar and starch contents and the amino acid composition of the toxic and non-toxic genotypes are almost identical. The levels of all essential amino acids, except for lysine, are comparable with the FAO reference protein for a growing child of 2-5 years of age.

These results imply that Jatropha kernel meal from the non-toxic genotype could be an excellent protein source for animals.

Toxicity of Jatropha

Jatropha plant contains variety of toxic and antinutritional compounds and its concentration varies with different parts of the plant. In brief, majority of the toxic effects were studied using aqueous or non aqueous extracts of Jatropha root, bark, leaf, stem, oil, and seed or from direct feeding of plant parts (Table 7). Majority of the organic solvent or aqueous extracts obtained from Jatropha plant contain phorbol esters and curcin respectively as the major toxic phyto-chemicals. Even though, information on the localization of antinutrients/toxic factors in aerial parts of Jatropha plant is limited, all parts of the plant are found to be toxic to both vertebrates and invertebrates. However, the severity and symptoms of toxicity varied with the extract types, dosage, administrative mode and sensitivity of the animal under investigation. The extracts from Jatropha plant parts or of plant products produced have a broad range of biological activities such as moluscicidal, piscicidal, insecticidal, antimicrobial and cytotoxic properties. The oral consumption of unprocessed Jatropha seeds or leaves are found to be toxic or lethal to animals such as rodents, pig, chicken, sheep, goat and calves. The main affected organs were liver, kidney, spleen, lungs, intestine and heart. Although limited information is available on the tox- icity of Jatropha towards humans, the oral, ocular or prolonged topical exposure should be avoided. The increased Jatropha cultivation in future and utilization of its agro-industrial prod- ucts/by-products may raise the frequency of contact with humans, animals, and other organ- isms. Thus, any uncontrolled disposal or spread of Jatropha plant products either in aquatic or terrestrial environment should be avoided.

Table 7. Toxicity of *Jatropha* species.

J. elliptica	Rhizome	Ethanol extract	Moluscicidal activity
	Tubercules	Ethanol extract	Toxic to rat (oral)
J. glauca	Leaves	Acetone extract from fresh leaves	Moluscicidal activity
		Chloroform extract from dry leaves	Moluscicidal activity
		Dry leaves	Toxic to goat (oral)*
	Fruit	Dry fruits	Toxic to goat (oral)*
J. gossypifolea	Bark	Aqueous extract from stem bark	Piscicidal activity
	Leaves	Fresh leaves	Toxic to sheep (oral)*
		Crushed liquid from fresh leaves	Anticoagulant activity
	Aerial parts (leaves and stems)	Ethanol extract	Toxic to mice and rats (intraperitoneal)
	Latex	Lyophilized powder	Piscicidal activity
J. multifida	Seeds	Raw seeds	Toxic to humans (oral)**
	Root (root bark, red root bark and root wood)	Hexane, ethyl acetate, chloroform and methanol extracts	Antibacterial activity
J. aceroides	Leaves	Dry leaves	Toxic to goat (oral)*
	Fruits	Dry fruits	Toxic to goat (oral)*
J. tanoresisi	Leaves	Ethanol (50%) extract	Toxic to rat (oral)
J. podagrica	Roots	Hexane and methanol extracts	Antibacterial activity
	Seed	Aqueous extract	Toxic to rat (intraperitoneal)
J. neopauciflora	Bark	Dichloromethane : methanol extract	Cytotoxic activity
J. macarantha	Sap	Aqueous extract	Toxic to mice (oral)

**force fed through stomach tube **accidental consumption*

Note: the table is adopted from our published review Devappa et al. (2010c). For cross reference please refer the review arti

Table 5. Toxicity of Jatropha plant species (adopted from our published review article, Devappa et al. 2010c).

Species	Plant parts	Test material	Properties
J. curcas	Plant	Chloroform and acetonitrile extracts	Moluscicidal activity
	Fruit	Methanol, chloroform and petroleum ether extract	Toxic to rat (pregnancy terminating effects)
	Seed	Powdered seed	Orally toxic to goat*, sheep*, calves* a (oral)**
		Powdered seed mixed in the diet	Toxic to chicken
		Raw or cooked seed	Toxic to rat (oral)
		Aqueous extract	Moluscicidal activity
		Methanol extract	Toxic to rats (intraperitoneal)
		Petroleum ether extract	Insecticidal activity
	Kernel meal	Mixed in the diet	Toxic to rat (oral); toxic to pig (oral)
	Defatted seed cake	Mixed in the diet	Toxic to rat (oral)
	Oil	Methanol and ethanol extracts	Moluscicidal and insecticidal activities; ha rabbit red blood cells; tumour promoting in mi
		Petroleum ether	Toxic to rats and rabbits (topical)
		-	Moluscicidal, ovicidal and insecticidal activit rats (oral and topical); pregnancy terminatin rats.
	Latex	-	Antiparasitic activity
	Aerial parts	Aqueous extract	Cytotoxic activity
	Leaves	Petroleum ether and benzene extracts	Insecticidal activity
		Methanol extract	Toxic to rats (oral); antischistosomal and c tivities
		Dichloromethane, methanol and hexane extracts	Antibacterial activity

Jatropha diterpenes

Since long time, *Jatropha* species are used in ethno medicines. This has led to identify the responsible bioactive molecules and finding possible pharmaceutical or agricultural applications. However, only few *Jatropha* species are explored for bioactive compounds, such as diterpenes, among others. Most of the diterpenes isolated were in search of new bio-control agents and their definite natural roles remain yet to be discovered. In brief, more than 65 diterpenes have been isolated from Jatropha and they exhibit diverse biologically activities (*in vitro*). The diterpenes such as jatrophone, jatrophatrione, spruceanol, cleistanthol, curcasones (A and B) and japodagrol possess antitumour activities. The hydroxy derivatives of jatrophones, jatropholones, curcasones, multifidone, jatrophalactam and faveline are cytotoxic. The caniojane derivatives, jatrogrossidione, hydroxy jatropholones, palmarumycin, jaherin and jatrogrossidentadion exhibited antimicrobial activities. Recent advances in analytical chemistry also led to the identification and comparison of novel chemical structure of these diterpenes, which could also be used as a template for the synthesis of new diterpene derivatives with enhanced functional and physical properties. In addition, phorbol type diterpenes (Jatropha factor C1-C6 and Jatropherol) isolated from Jatropha species has rodenticidal, piscicidal, moluscicidal and insecticidal activities, indicating their potential as bio-control agents in agriculture. The abundance and novelty of diterpenes present in Jatropha species could form a new "stock" for the pharmaceutical industries. Future expansion of Jatropha plantation could generate a huge amount of raw materials for both biodiesel and pharmaceutical industries.

The main toxic compounds present in Jatropha curcas seeds

Several antinutritional factors are present in the *Jatropha curcas* seed: protease inhibitors, lectins, also known as curcin, saponins, phytates and phorbol esters.

Protease inhibitors are peptides capable of binding to the pancreatic proteolytic enzymes (trypsin and chymotrypsin), making them inactive. Trypsin inhibitors interfere with the physiological process of proteins digestion by disabling the action of pancreatic proteolytic enzymes in non-ruminants, leading to severe negative disorders of the pancreas and a corresponding reduction in the digestibility of proteins in the diet, followed by a growth decrease. Trypsin inhibitors are heat-labile and can be completely denatured when exposed to high temperature (moist heating at 121°C for 25 min).

Lectins are a kind of toxalbumin, which, isolated from the seeds of *J. curcas*, were designated as "curcin". More recently curcin was reported to be a single chain protein and more precisely a type-I ribosome-inactivating protein. Common in plants, it is often called haemagglutinin, due to its ability to cause agglutination of erythrocytes in various species of animals, having high binding capacity to specific carbohydrates, mainly in cells of the duodenum and jejunum, causing serious damage to the intestinal wall. Clinical signs such as intense inflammation with destruction of epithelial cells, oedema, hyperaemia, haemorrhage in lymph tissue, fatty degeneration and necrosis of the liver, myocardial and vascular system injuries are found when this substances ingested. Curcin from Jatropha seed is likely to be inactivated by the processes used to produce seed meal.

Saponins (lower in *J. curcas* than that reported for soybean seed) are glycosides or steroids, characterized by a bitter taste, the ability to form foam in aqueous solutions, causing haemolytic disorder and complex changing in steroids. Antinutritional effects are also related to changes in the permeability of the intestinal mucosa, inhibiting the transport of some nutrients. However, these saponins do not possess haemolytic activity. Because of the high solubility of saponin in water, the removal by aqueous extraction can be done for most components that contain high levels of saponin.

Phytate or **phytic acid** are constituents not specific to *J. curcas*, but are in all cereals and oilseed meals such as soybean, cotton, rapeseed and peanut. Phytate acts by forming a complex with transition elements such as zinc, iron, calcium and manganese in the gastrointestinal tract, preventing their absorption, reducing the availability of minerals and affecting protein digestibility by the formation of the complex protein-phytic acid. A moist heat treatment can minimize the damage, reducing the concentration of phytic acidin feed and the supplementation of enzyme phytase in the diet can neutralize negative effects of high phytate levels.

Phorbol esters (phorbol-12-myristate 13-acetate) are identified as the major toxic antinutritionals in Jatropha. They are a group of diterpene esters, present in plant species of the *Euphorbiaceae* and *Thymelaeaceae* families and contained in relatively high concentrations in the seed kernels of *J. curcas*, ranging generally from 0.87 to 3.32 g/kg. While the other antinutrients present in *J. curcas* seed are in negligible amounts or easy to inactivate by moist heating, which normally occurs during the seed meal production process, the phorbol esters (PE) are heat stable and can withstand temperatures up to 160°C for 30 min, being completely degraded only by

a stripping/deodorization process at 260°C with 3 mbar pressure and 1% steam injection. For the above reasons PE are considered the main toxicity problem to be solved to allow a useful livestock utilization of *J. curcas* seed cake because, even at very low concentrations they show severe toxicological effects in animals, both ruminants and monogastrics.

The case of phorbol esters to be the main toxic principle

Phorbol esters were absent in kernel meal from the non-toxic genotype but were present in high concentrations in the kernel meal from the toxic genotype. Phorbol esters, diterpenes of phorbol type, cause severe toxic symptoms in livestock. At least six phorbol esters are present in Jatropha seeds. The phorbol esters are reported to mimic the action of diacylglycerol, an activator of protein kinase C, which regulates different signal transduction pathways. Interference with the activity of protein kinase C affects a number of processes including phospholipid and protein synthesis, enzyme activities, DNA synthesis, phosphorylation of proteins, cell differentiation and gene expression. They are also co-carcinogens and have purgative and skin-irritant activities. In humans, accidental poisoning by Jatropha seeds has been reported to elicit giddiness, vomiting and diarrhoea. Mortality has also been reported in a number of animal species, *e.g.* mice, chicks and goats. Phorbol esters are heat stable and, hence, heat treatment is not effective to detoxify kernel meal from the toxic genotype. On the other hand, trypsin inhibitor and lectins are heat labile and can be destroyed by moist heating. Consumption of unroasted seeds of non-toxic Jatropha is known to produce discomfort in humans, and this could be due to the presence of trypsin inhibitor and lectins. The roasting treatment has been found to reduce the level of trypsin inhibitor completely. The lectin activity decreased by approximately 50% on roasting and the phytate level remains unchanged. Consumption of large amounts of kernels from the roasted seeds might produce discomfort due to the remaining lectin activity.

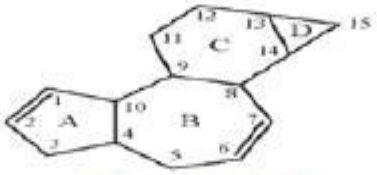
Phorbol esters: chemistry, biological activity and potential applications

Phorbol esters are not new to life science research. The phorbol ester has been widely used to understand the molecular mechanisms in cancer research, immunology, toxicology and nutrition. These compounds have fascinated many researchers from basic science to molecular

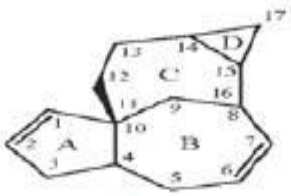
science resulting in more than 22,000 research articles published over the past 40 years (www.scopus.com search key: phorbol esters). The complexity and richness of the studies are due to the multiple bioactivities of phorbol esters. The majority of the articles describe phorbol esters as toxic and as tumour promoters. More recent studies also portray phorbol esters as anticancer agents or as potential medicinal compounds (Goel *et al*., 2007; Wender *et al*., 2008). The following sub-sections, provides a brief overview of the chemical and biological properties of phorbol esters.

Distribution and chemical structure of phorbol esters

Phorbol and its related compounds are isolated from various natural sources. These esters are widely distributed in plant species of the families *Euphorbiaceae* and *Thymelaceae*. Examples of plants from which phorbol compounds have been isolated include among others, *Euphorbia Fischeriana, Homalanthus nutans, H. acuminatus, Neoboutonia melleri, Excoecarcia agallocha, Croton califonicus, Croton tiglium, Sapium indicum, S. japonicum, E. frankiana, E. cocrulescence, E. ticulli, C. spareiflorus, C. ciliatoglandulifer* and *Jatropha curcas* (Beutler *et al*. 1989; Goel *et al*., 2007; Haas *et al*., 2003). Zayed *et al*. (1977) have also reported the presence of phorbol epoxides with the structure R2 (Figure 1).



Structure R1 (tigliane)



Structure R2 (tigliane epoxide)

Figure 1. Basic structure of phorbol esters

The isolation of phorbol compounds from natural sources often requires great skill. The term "phorbol" refers to a group of compounds belonging to closely related families of diterpenes with polycyclic structural formulas (Figure 1). The ring ABCD can be attached to one or more substituents. Generally, substituents include hydroxyl, heteroalkyl, alkoxy, alkyl, arylalkoxy, hydroxyalkyl, acyloxy, aldehyde groups or combinations thereof. The numbering of atoms in the basic polycyclic structure is shown in the following Figure 1. Phorbol is more correctly described as a diterpene having a tigliane skeleton. The tigliane skeleton consists 5-membered ring A on the left trans linked to the 7-member ring B. Ring C is 6-membered and cis linked to the cyclopentane ring D. The compound phorbol was first isolated from *Croton tiglium* seed oil in 1934 (Bohm *et al*., 1934). Based on the generally accepted nomenclature system, phorbol is 4, 9, 12, 13, 20-pentahydroxy-1,6-tigliadien-3-one. The term phorbol ester applies to diversely oxygenated and hydroxylated tiglianes in different esterified forms. The placement of an OH group in 7 membered ring (at C4 position) makes the phorbol an active (ß) or inactive (α) type, which results in spatial re-arrangement of ring D and precludes the activation of PKC (Protein kinase C) and other structurally similar phorbol ester receptors. The inactive "α" phorbol esters have similar physicochemical properties, especially lipophilicity, as the active "ß" phorbols, but are unable to activate PKC due to conformational shifts (Figure 2) (Silinsky & Searl, 2003).

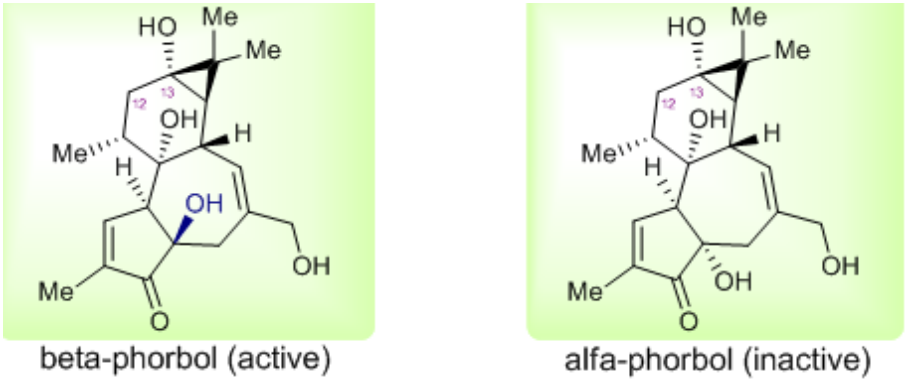


Figure 2. Phorbol esters and their confirmation responsible for bioactivity.

The most studied phorbol ester is phorbol 12-myristate-13 acetate (PMA) (synonym: 12-ter-tradeconoylphorbol-13-acetate (TPA) (commercially available). PMA has been isolated from *Croton tiglium* oil. Phorbol esters are easily soluble in most organic solvents such as ethanol, methanol, dichloromethane, and dimethyl sulfoxide, among others and are found to be sensitive to oxidation (Schmidt & Hecker, 1975). PMA is the most potent tumour promoter known to date and is widely used in scientific research. Phorbol esters can be extracted by alcohol/organic solvent extraction or partition methods. Further purification can be carried out using chromatographic techniques, including, HPLC, gel exclusion chromatography. Similarly, many synthetic methods for producing parent phorbol compounds and their derivatives have been reported. Although more than 60 different types of natural phorbol esters have been reported, very few have been studied in detail with respect to their biological activity. The chemical structures of phorbol esters are described in detail by Haas *et al*. (2003).

Mechanisms of phorbol ester bioactivity

Phorbol esters exhibit multiple biological activities including the promotion of tumours. The most studied activity of the phorbol is its activation of PKC (Protein kinase C), which plays an important role in signal transduction pathways and regulates cell growth and differentiation (Clemens *et al*., 1992; Nishizuka, 1992). Phorbol esters are analogues of diacylglycerol (DAG), which is a secondary messenger in one of the main cellular signal transduction pathways (Figure 3a and 3b).

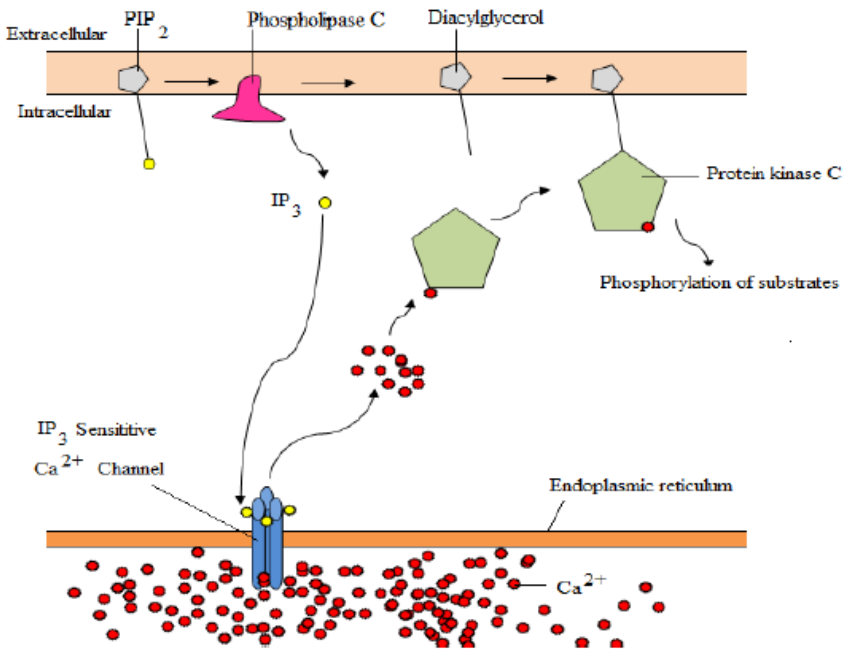


Figure 3a. Signal transduction pathway involving protein kinase C

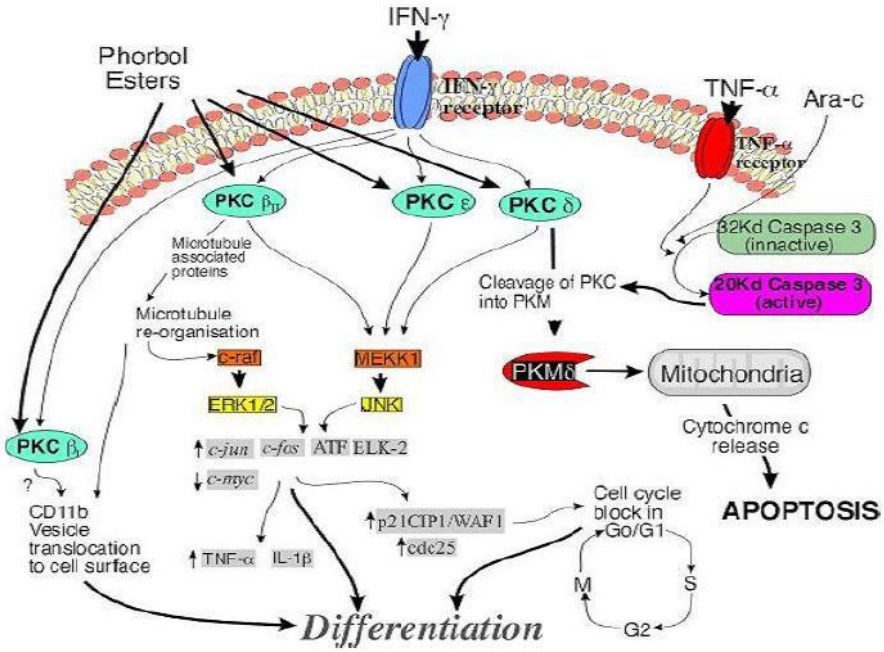


Figure 3b. Molecular effects of phorbol esters in cell signal transduction

(Source: http://www.spacedu.com/protein_kinase_c_experiments.htm).

Figure 3a, 3b. Phorbol esters effects in cellular signal transduction pathways.

In brief, the enzyme phospholipase C (PLC) (a membrane-bound enzyme) catalyses the hydrolysis of phospholipid PIP2 (phosphatidyl inositol-bisphosphate) to produce diacylglycerol (DAG) and inositol trisphosphate (IP3). The inositol trisphosphate (IP3) diffuses into the cytosol, but DAG due to its hydrophobic properties remains bound in the plasma membrane. These IP3 molecules stimulate the smooth endoplasmic reticulum to release calcium ions, which act as a cofactor and facilitate the translocation of PKC from the cytosol to the plasma membrane. The DAG activates the enzyme PKC which catalyses the phosphorylation of other proteins that are involved in signal transduction. The biological activities of phorbol esters are structure dependent. The translocation of PKC to the cell membrane depends on the hydrophobicity of the phorbol ester side chain and its ability to incorporate itself into the membrane (Bertolini *et al*., 2003). The data obtained from phorbol 12- myristate-13 acetate (PMA), its derivatives and analogues revealed that initial binding of a phorbol ester takes place at C1 domain of PKC.

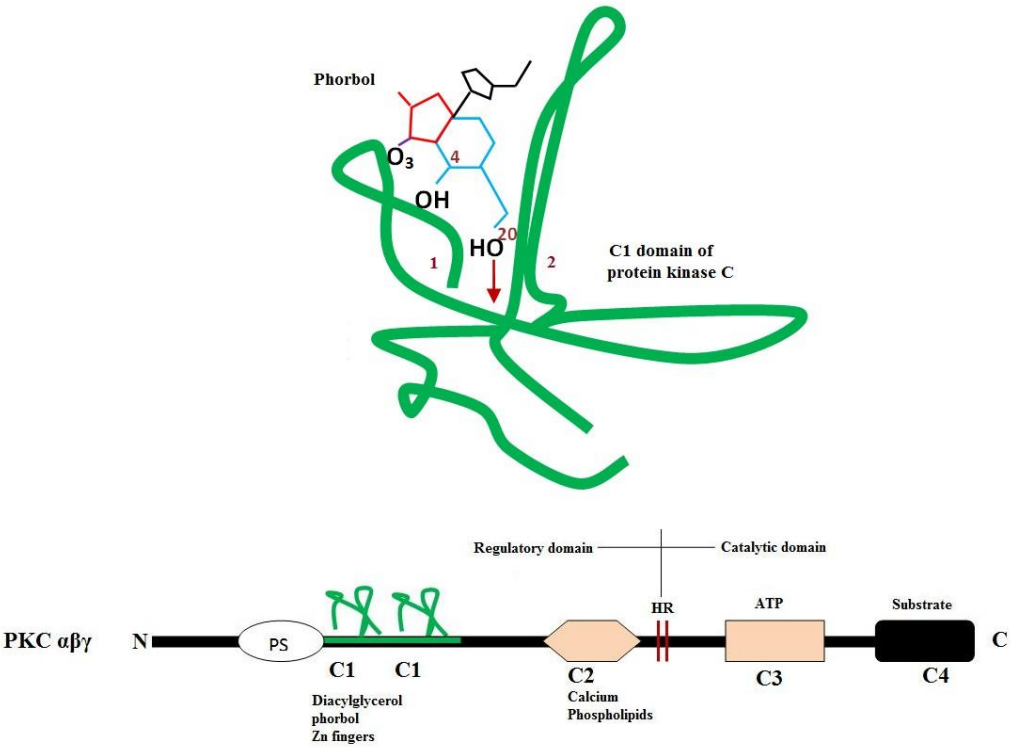


Figure 4. Mechanism of phorbol ester binding to protein kinase C domain.

Biological activity of phorbol esters

The C1 domain contains two β sheets separated by water-filled cavity (Figure 4). Phorbol esters displace water and fit into this cavity so that the phorbol esters become attached to the PKC via the oxygens present at the C3, C4 and C20 of the phorbol ester. The binding of phorbols in the C1 domain provides hydrophobicity to the complex and allows it to get inserted and anchored into the membrane without conformational change. After insertion into the membrane, the cat- alytic domain of PKC is activated and phosphorylation takes place after binding of appropriate substrates to the C4 domain (Figure 4). The hyper activation of PKC by phorbol esters (DAG analogue) results in uncontrolled differentiation, thus amplifying efficacy of carcinogens.

The phorbol esters exhibit biological activities such as tumour promotion, platelet aggregation, apoptosis, cell differentiation and other metabolic effects. Among these, tumour promotion by phorbol esters have been most studied (Goel *et al*., 2007). A tumour promoter is a compound that in classical studies of carcinogenesis is able to increase the likelihood of tumour formation after the application of a primary carcinogen. However, it does not induce tumour formation when applied alone. Non-tumour promoting phorbol esters all have at least one of the biological activities of phorbol compounds in general such as binding to phorbol receptors, but do not have tumour promoting properties. Non-tumour promoting phorbol compounds include 12-de- oxyphorbol 13-acetate (prostratin), 12-deoxyphorbol 13-propanoate and 12-dexoxy phorbol13- phenylacetate (Xu *et al*., 2009). Toxic phorbol esters are skin irritants and exhibit mammalian skin inflammatory response releasing increased histamine, prostaglandins and cytokines (Fig- ure 5).

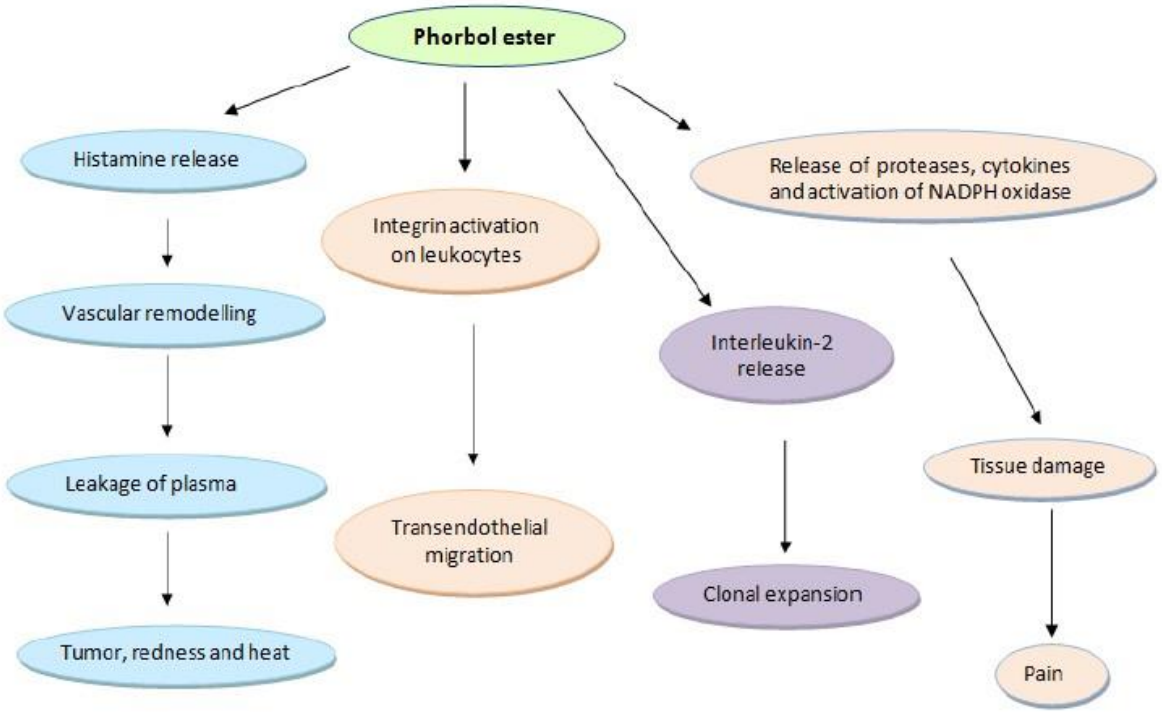


Figure 5. Major biological activity of phorbol esters (adopted from Goel et al., 2007).

Figure 5. Major biological activity of phorbol esters.

Weinstein *et al*. (1979) have reported that phorbol esters exhibit generalized cellular membrane changes such as changes in membrane morphology, cell surface fucose glycopeptides, cell adhesion properties and membrane fluidity. There are also increased levels of ornithine decarboxylase and plasminogen activator. The phorbol esters block the G2 phase of the cell cycle due to their effect on multiple targets within a single cell causing cellular differentiation. Phorbols also affect terminal cellular differentiation, which cumulatively results in production of tumours. Phorbol esters are also potent human blood platelet aggregators exhibiting aggregation at exceptionally low levels (0.3 μM = 50% aggregation). The platelet aggregation assay has been suggested to be a choice for initial screening of tumour promoting substances (Brynes *et al*., 1980; Zucker *et al*., 1974). The phorbol esters affect many enzyme activities by interacting with PKC such as reduction in phosphoenol pyruvate carboxykinase in H4IIE cell lines, a key enzyme in gluconeogenesis (Chu & Granner, 1986).

Phorbol esters: the good, the bad and the ugly

As with any other chemical, the toxicity of the compound depends on dosage, mode, and dura-

tion of exposure, genetic factors (animal, age, weight). Generally, the toxicity increases with the concentration of the toxic compound to which an organism is exposed. However, at lower nontoxic dosages these chemicals can sometimes exhibit beneficial properties for example cytotoxic, antitumour or anti HIV properties (Goel *et al*., 2007). Similarly, phorbol esters also possess toxic and beneficial biological activities, acting as a double-edged sword. At high doses they exhibit toxicity that can be observed in microorganisms and even in higher animals. The over dosage of phorbol esters are found to be lethal towards higher animal either by oral or topical exposure (Gandhi *et al*., 1995). Therefore, the presence of phorbol esters in feed ingredients is deleterious. However, at lower dosages phorbol esters exhibit antitumour properties *in vitro* thereby acting as potentially beneficial compounds (Wender *et al*., 2008). Not all phorbol esters are toxic. Their activity and potency vary from one type of phorbol ester to another. In addition, these chemicals in a crude form (extracts) are effective in controlling microbes and pests of agricultural interest, suggesting that they may have application as biological control agents. The purified phorbol esters could also be converted or transformed chemically into nontoxic compound with beneficial activities such as prostratin. Prostratin has been found to be a promising anti-HIV agent. Thus, the beneficial effects of phorbol esters could be exploited depending on the application.

Phorbol esters from Jatropha curcas

Depending on the presence or absence of phorbol esters, Jatropha species are classified as toxic and nontoxic genotypes respectively. The most studied Jatropha species, *J. curcas*, contains phorbol esters about 1-3 mg/g in defatted kernel meal or 3-6 mg/g in oil.

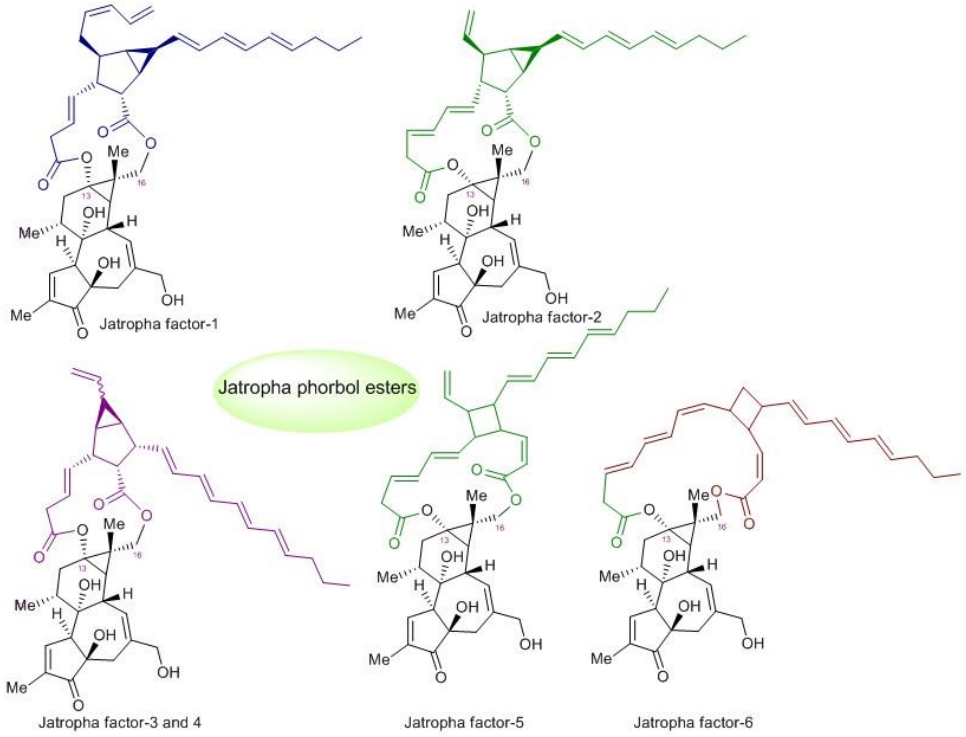


Figure 6. Phorbol esters from *Jatropha curcas* oil.

There are six different types of phorbol ester reported in Jatropha seed (Haas *et al*., 2002). The chemical structure is shown in Figure 6.

They named the isolated fraction as Jatropha factors C1 to C6 (Figure 6). Factor C1 had typical bicyclohexane unit, a vinyl group, nonatrienyl residue, and single carbonyl ester chain at C-12. Factor C2 differed from factor C1 in length of carbon chain (C-6 in factor C1 and C-8 in factor C2), length of ester chain connecting bicyclohexane unit with C-13 (C5 in factor C1 and C7 in factor C2), and the configuration at C-6 and C-8 in factors C1 and C2, respectively. Jatropha factors C3 and C6 were reported to have cyclobutane ring. Factor C6 differed from factor C3 in having trisubstituted cyclobutane unit rather than tetrasubstituted unit of factor C3 and in length of ester chain at C-13 of phorbol unit. The absolute stereochemistry and relative configuration of the cyclobutane unit could not be determined.

Jatropha factors C4 and C5 were isolated as epimers as they were not separated by chromatography. These two units differed from factor C1 in length and position of carbon chains and orientation of bicyclohexane unit relative to phorbol. All the intramolecular diesters were reported to build from two separated monoester groups and the two dicarboxylic groups bound to the OH-13 and OH-16 of the phorbol moiety.

Activities of Jatropha curcas phorbol esters in various bioassays

Isolated phorbol ester rich fraction (PEEF) was used to evaluate the activity of PEs using three aquatic species based bioassays snail (*Physa fontinalis*), brine shrimp (*Artemeia salina*), daphnia (*Daphnia magna*) and microorganisms. In all the bioassays tested, increase in concentration of PEs increased mortality with an EC50 (48 h) of 0.33, 26.48 and 0.95 mg L^{-1} PEs for snail, artemia and daphnia, respectively. Among the bacterial species tested, *Streptococcus pyogenes* and *Proteus mirabilis* were highly susceptible with a minimum inhibitory concentration (MIC) of 215 mg L^{-1} PEs; and Pseudomonas putida were also sensitive with MIC of 251 mg L^{-1} PEs. Similarly, Fusarium species of fungi exhibited EC50 of 58 mg L^{-1} PEs, while *Aspergillus niger* and *Curvularia lunata* had EC50 of 70 mg L^{-1}. The snail bioassay was most sensitive with 100% snail mortality at 1 mg of PEs mL^{-1}. In conclusion, snail bioassay could be used to monitor PEs in Jatropha derived products such as oil, biodiesel, fatty acid distillate, kernel meal, cake, and glycerol or for contamination in soil or other environmental matrices. In addition, PEs with molluscicidal/antimicrobial activities could be utilized for agricultural and pharmaceutical applications.

Ocular and dermal toxicity of Jatropha curcas phorbol esters

Upon topical exposure to reconstructed human epidermis and human corneal epithelium, the toxic Jatropha oil exhibited severe histological alterations and inflammatory responses, while these responses were minor with the nontoxic Jatropha oil. Similarly, the purified PE also elicited severe histological alterations and inflammatory responses, suggesting their role in Jatropha toxicity. Thus, the direct contact with toxic Jatropha oil or phorbol ester containing Jatropha products should be avoided. It is advised to use the protective gloves and glasses when handling phorbol ester containing Jatropha products.

Utilization of Jatropha meal and protein isolate from toxic genotype in animal nutrition

Tests results of use of detoxified Jatropha kernel meal and protein isolate in diets of farm animals

The increased global demand to meet the protein requirement in animal nutrition and future energy needs has given impetus to the search for alternative plant sources that do not compete with human nutrition and at the same time have high oil content.

Jatropha seeds may be a good alternative with respect to their multipurpose features such as high adaptability for cultivation, applicability of seed oil for biofuel production, and generation of productive value-added coproducts. The plant has potential to enhance both food and energy security as well as contribute to land reclamation and prevention of land degradation. Jatropha proteins have interesting nutritional and biochemical properties. High protein content and their digestibility and good amino acid composition make Jatropha proteins a promising source for incorporation into the diets of ruminant and monogastric animals including fish. A major drawback for poor utilization of Jatropha proteins in animal nutrition has been the presence of antinutritional factors and particularly of phorbol esters. However, a detoxification process will pave the way for using Jatropha kernel meal and protein isolates in livestock nutrition.

Recently, in many laboratories Jatropha kernel meal and protein isolate have successfully been detoxified. The detoxified Jatropha kernel meal (DJKM) and detoxified protein isolate (DPI) prepared from screw-pressed cake have been utilized in animal nutrition with excellent growth performance and no toxic effects at blood and tissue levels. The DJKM and DPI have high protein contents (60 and 90%, respectively) and excellent amino acid composition, and these could replace at least 50% of the protein contributed by the high-quality fish meal (65% protein) in standard fish diet.

Impact of feeding detoxified Jatropha kernel meal on common carp

Kumar *et al.* (2011) performed experiments wherein 50 and 75%, and 50 and 62.5% of fish-meal protein was replaced by DJKM, with synthetic lysine added in the DJKM containing diets. Based on visual observations during feeding time, acceptability and palatability of the DJKM-

based feeds was similar to the control diet. High inclusion (>50% replacement of fishmeal protein) of the detoxified meal resulted in reduced protein utilization, measured as protein efficiency ratio and protein productive value (Kumar *et al*., 2011, 2010).

These results showed that 50% replacement of fishmeal protein by DJKM in common carp diet met the dietary demands for protein and energy. Based on these results it is concluded that 50% of the fishmeal protein can be replaced by DJKM in common carp diets without compromising growth and nutrient utilization.

However, >50% replacement of fishmeal protein by DJKM leads to significantly lower growth and higher feed conversion ratio (feed/body mass gain) in common carp, which could be attributed to factors such as:

- lower digestibility of protein and energy in the diets, leading to lower protein and energy availability from DJKM;
- the DJKM contains large concentrations of antinutrients such as phytate and non-starch polysaccharides (NSPs), and these could adversely affect feed utilization;
- the digestibility of synthetic lysine, which was added as a supplement to the diets, may be less than that of the natural amino acid present in the feed ingredients.

Retention of nutrients in the whole body

The efficiency with which nutrients and energy are retained from feeds provides a useful assessment of the efficiency of nutrient utilization from diets (Booth & Allan 2003; Cho & Kaushik, 1990; Glencross *et al*., 2004). Feeding trials performed by Kumar, *et al*., (2011 2010) showed that inclusion of DJKM in a common carp diet exhibited significantly higher lipid deposition in the whole body than in the control group. When compared with fishmeal, feeding DJKM to common carp led to higher whole body crude protein content, showing that DJKM contains optimum digestible energy and has a balanced amino acid profile ideal for fish growth. Dietary inclusion of DJKM reduced the cholesterol level in plasma and muscle when compared with the fish meal fed group (Kumar *et al.*, 2010b). Further, fibre and antinutritional factors (NSPs and phytate) reduce absorption of total fat, including cholesterol, when these factors are increased in the diet (Hansen, 2009; Krogdahl *et al*., 2003). Faecal excretion of steroids (bile acids) is the major pathway for elimination of cholesterol from the body (Hansen, 2009).

Energy budget and metabolic efficiency

Growth and production can be described in terms of partition of dietary energy between catabolism as fuels and anabolism as storage in tissues. Makkar and Becker (2010) reported that common carp fed DJKM and fishmeal-based diets exhibited similar values for routine metabolic rate.

These observations suggest that energy requirement for digestion and absorption of nutrients from DJKM and fishmeal are similar, and that DJKM is a promising good quality protein source for incorporation in feed for common carp.

Common carp health

Haematological, biochemical and histological measurements are an integral part of evaluating the health status of commercially important fish. The activities of alkaline phosphatise (ALP) and alanine transaminase (ALT) in blood are used as indicators of liver cell condition. Usually, the level of ALP and ALT rises in blood during acute liver damage (Goel *et al*., 1984). Feeding DJKM and fishmeal did not change levels of ALP and ALT activity in the blood (Kumar *et al.*, 2010b; Kumar, 2011). Other health-related blood parameters, such as blood urea nitrogen, total bilirubin and creatinine contents, which are indicators of liver, kidney and gill function (Stoskopf, 1993; Tietz, 1986) were also in the normal range (Kumar *et al.*, 2010b). This suggested that the liver, kidney and gills of the common carp were in a normal functional condition in the DJKM-fed groups. Also when common carp were fed DJKM as a protein source (Kumar *et al.*, 2010b; Kumar, 2011), haematology (haematocrit, haemoglobin and red blood cell count) values were within normal ranges. Blood protein is considered a basic index for health and nutritional status in fish (Martinez, 1976). Albumin, globulin and total protein concentrations in blood were within the normal range for DJKM-fed groups (Wedemeyer & Chatteron, 1970; Sandnes *et al*., 1988). Also DJKM diets exhibited no abnormal changes in intestine and liver (Kumar *et al.*, 2010b). The intestinal mucosa was well developed, no morphological alteration was found, and the intestinal mucosa appeared to be normal for common carp. Liver also showed no pathological alteration or signs of steatosis or hepatic lipidosis in the DJKM-fed group (Kumar *et al.*, 2010).

Based on the above findings, it was concluded that DJKM can replace 50% fishmeal protein without comprising growth, nutrient utilization or health of the fish.

Use of detoxified Jatropha kernel meal in rainbow trout (Oncorhynchus mykiss) diet

Impacts on growth and feed utilization

The utilization of detoxified Jatropha kernel meal (DJKM) as a protein source in a carnivorous fish species, rainbow trout (*Oncorhynchus mykiss*), was investigated (Kumar *et al*., 2011b). In this study, 50% (J50) and 62.5% (J62.5) fishmeal protein was replaced by DJKM. Palatability and acceptability of DJKM-based diets were similar to that of the fishmeal-based diet. Growth performance, nutrients and energy digestibilities were similar for control and J50 group, but were higher than for J62.5 group. Feed conversion ratio, protein efficiency ratio, protein productive value and energy retention were similar for control and DJKM-fed groups.

Impacts on health of fish

Metabolic enzyme (ALP and ALT) activities, metabolites (urea nitrogen, total bilirubin and creatinine) and ion concentrations in blood were in the normal range in the groups in which 50% and 62.5% of fishmeal protein was replaced by DJKM. Blood parameters such as red blood cell (RBC) and white blood cell counts, haematocrit and haemoglobin level were also not affected by dietary treatments (Kumar *et al*., 2011b) and their ranges were also in the normal range for healthy trout. After feeding DJKM as a protein source to the rainbow trout, no signs of histopathological lesions were observed in the organs (Kumar, 2011). The gastric glands were well developed and the epithelium lining the luminal surface that consists of highly co-lumnar cells and produces protective mucous was not altered. The case for the branched tubular glands was similar, as they were also well developed. There was no change in the shape and cellular morphology of pepsin- and hydrochloric acid-producing cell-types (oxyntopeptidic cells), indicating no leucocyte immigration and therefore no signs of inflammation (Kumar, 2011). In addition, there was no alteration in intestinal loops, pyloric appendices, the terminal hind gut, and the villi of the appendices or terminal intestine. There was also no sign of hepatic steatosis or lipidosis in rainbow trout when fed with DJKM as a protein source.

Conclusively, DJKM can replace 50% fishmeal protein without compromising the growth, feed utilization and health of rainbow trout.

Use of detoxified Jatropha kernel meal as a protein source in white leg shrimp feed

Greater growth response and nutrient utilization were observed in DJKM-fed groups (25 or 50% fishmeal protein replaced by DJKM) compared with fishmeal-fed group in white leg shrimp (*Litopenaeus vannamei*) (Harter *et al.*, 2011). The DJKM protein in combination with fishmeal protein gave excellent nutrient and energy digestibility, leading to higher growth performance and nutrient utilization (Harter *et al.*, 2011). These results, along with the amino acid composition of the diets tested, indicated that the requirements of shrimp (Akiyama & Tan, 1991; Van Wyk, 1999) for amino acids, were met. In the whole body of the shrimp, there was no significant effect on lipid deposition after feeding DJKM, whereas protein and energy deposition were significantly higher in the control group compared with DJKM-fed groups (Harter *et al.*, 2011). Reduction in plasma cholesterol level in shrimp as dietary fishmeal levels decreased and DJKM levels increased was a consequence of the reduced amount of cholesterol available in the diet (Harter *et al.*, 2011).

Overall, growth performance and nutrient utilization in white leg shrimp for DJKM-fed groups were better than for the control group, which suggests that white leg shrimp, can efficiently use DJKM as a good quality protein source.

Use of detoxified Jatropha curcas protein isolate in common carp feed

Impacts on feed intake and growth performance

Kumar *et al*. (2011) observed that detoxified Jatropha protein isolate (DJPI)-based diets had excellent palatability for common carp and there was no wastage of feed during the experiment. Common carp fed a diet containing DJPI (50% replacement of fishmeal protein) grew significantly better than those on the fishmeal-based control diet (Kumar *et al.*, 2011; Nepal *et al.*, 2010). However, a higher level (75% replacement of fishmeal protein) of DJPI exhibited

growth performance similar to that with the control diet (Kumar *et al*., 2011d; Nepal *et al.*, 2010).

Impacts on digestive physiology

DJPI in combination with fishmeal protein showed excellent nutrient and energy digestibilities in common carp (Kumar *et al.*, 2011). Compared with fishmeal protein, DJPI had similar apparent protein and lipid digestibility, which could be attributed to the absence of a trypsin inhibitor and lectin and the addition of phytase to mitigate the effects of phytate, if any. Dietary inclusion of DJPI did not alter the intestinal digestive enzyme (amylase, protease and lipase) activities.

Impacts on nutrients retentions

Inclusion of DJPI in feed exhibited higher lipid retention in the whole body of fish compared with control (fishmeal) fish. Protein deposition in the whole body of common carp was more pronounced in DJPI-fed groups compared with the fishmeal-fed group, which concurs with the higher value of protein productive value in the former group. Interestingly, Kumar *et al*. (2011) found that protein retention in the body of common carp was significantly higher in DJPI-fed groups than the control group. This finding reveals that DJPI-containing diets have optimum digestible energy and a balanced amino acid profile for optimum growth and optimum nutrient deposition in fish.

Impacts on biochemical parameters and haemato-immunology

Feeding DJPI as a protein source significantly decreased cholesterol level in plasma and muscle compared with the control group, however these values were in the normal range for healthy carp (Ghittino, 1983). Albumin and globulin concentrations in blood for DJPI-fed groups were within the normal range (Wedemeyer & Chatterton, 1970; Sandnes et al., 1988). Feeding DJPI as a protein to common carp exhibited levels of metabolic enzyme (ALP and ALT) activities

similar to those in the control group, suggesting normal organ function and absence of toxic factors in DJPI (Nepal *et al.*, 2010). Blood glucose concentration was unaffected by dietary inclusion of DJPI in common carp (Nepal *et al.*, 2010).

Conclusions regarding the use of detoxified kernel meal and detoxified protein isolate from Jatropha curcas as aqua feed.

Effects on growth and nutrient utilization

Detoxified Jatropha kernel meal (DJKM), non toxic Jatropha kernel meal (NTJKM) and detoxified Jatropha protein isolate (DJPI) can replace 50, 62.5 or 75% fishmeal protein, respectively, without compromising growth performance and nutrient utilization in fish. In addition, DJKM can also replace 50% fishmeal protein without any adverse effects on growth and nutrient utilization in shrimp. High inclusion (>50% fishmeal protein replacement) of DJKM decreases the efficiency of conversion of feed to body mass. No such effects were seen on using DJPI in common carp diets. Increased DJKM inclusion (>50% fishmeal protein replacement) in diets caused a significant lowering of protein, lipid and energy digestibility. No such effects were observed when DJPI was used in common carp diets.

Effects on energy budget

Feeding DJKM and NTJKM to common carp and Nile tilapia respectively did not change the major components of the energy budget (routine metabolic rate, heat released and metabolizable energy) compared with fishmeal and soymeal fed groups. These results showed that, as dietary protein sources, DJKM and NTJPKM can be efficiently utilized for growth by common carp and Nile tilapia respectively, and as good as soymeal and fishmeal.

Effects on clinical health parameters and gut health

No mortalities and unaffected haematological values suggested that the fish were in normal health. ALP and ALT activities, urea nitrogen, bilirubin and creatinine concentrations in blood

were in the normal ranges, showing no liver or kidney dysfunction. The plasma nutrient levels measured gave no indications of stress, but increasing the level of plant protein in the diet decreased plasma cholesterol. A decrease in muscle cholesterol level is also expected, which could be considered good for human health. Histopathological evaluation showed no damage to stomach, intestine or liver of common carp or rainbow trout.

Use of detoxified Jatropha curcas kernel meal in poultry feed

Soybean and canola meals (i.e. rapeseed meal) are the major protein meals used worldwide in poultry feed (USDA, 2010). However, SBM competes with human food and there is a need to search for alternative plant-protein sources for poultry feed. The nutrient and energy concentrations of DJKM compare well with that of SBM, with a higher content of EAAs (except lysine). Boguhn *et al.* (2010) evaluated the nutritional quality of DJKM in turkeys (3-week-old) by including at levels of 0% (control), 10% (J10) or 20% (J20) into a basal diet based on maize, SBM and wheat gluten, at the expense of maize starch. Body mass gains were 42, 54 and 57g/day for control, J10 and J20 groups respectively. Feed efficiency (gain:feed ratio) was significantly higher in DJKM fed groups (0.81 and 0.82 *vs.* 0.70). Precaecal amino acid digestibilities of amino acid from DJKM varied from 0.48 (cystine) to 0.91 (methionine). Mean digestibility of the non-essential amino acids was 80%, while that of EAAs was 83%.
Considering growth performance, nutrient utilization and amino acid digestibility of DJKM, it can be concluded that DJKM is valuable protein source for turkeys.

Use of detoxified Jatropha curcas kernel meal in pig feed

The most commonly used source of supplemental protein in diets for non-ruminants is SBM because of its excellent amino acid profile and dependable supply. In a typical pig diet, soybean supplies about 50% of the protein and amino acids and about 25% of the metabolizable energy. Wang *et al.* (2011) investigated the effects of replacing SBM by detoxified *J. curcas* kernel meal (DJKM) in the diet of the growing pig. The DJKM protein replaced 25 or 50% of SBM protein in the diets, and the DJKM-containing diets were supplemented with lysine (~2% of DJKM inclusion). There were no significant differences in growth performance and feed utili-

zation on substituting 25 or 50% of SBM protein with DJKM. These results show that the nutrient value of a DJKM-supplemented diet containing additional lysine is comparable with that of SBM for growing pigs. Dietary inclusion of DJKM did not affect carcass weight, dressing percentage, back fat thickness or visceral organ weight and its ratio to body weight when compared with the control group. In addition, glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase and ALP activities and the concentrations of albumin, urea, glucose and triglycerides in serum did not change in growing pigs. There were no histopathological changes in liver and kidney of growing pigs fed DJKM diets (Wang *et al.*, 2011).

The above data show that incorporation of DJKM had no ill effects on health, and it can replace 50% soy meal protein in diets of growing pigs.

Use of *Jatropha curcas* meal of a non-toxic Jatropha genotype

Potential of Jatropha meal from non-toxic genotype in animal nutrition

The meal from the nontoxic genotype is free of PE, but it contains trypsin inhibitor, lectin, and phytate at the same levels as the meal from the toxic genotype. The nutritional quality of the nontoxic Jatropha meal, after heat treatment (to inactivate trypsin inhibitor and lectin), evaluated in fish (carp) and rat models was found to be very high. The meal or the protein isolate obtained from the nontoxic genotype, after heat treatment, could be an excellent protein-rich ingredient in feeds of ruminant and monogastric animals including fish. Heat-treated (121°C at 66% moisture for 15 min) and unheated *J. curcas* kernel meals of a non-toxic variety were used as protein sources for common carp diet. The heat treatment was done to inactivate trypsin inhibitor and lectins. Similar growth performances were observed for both the groups, suggesting no physiological relevance of heat-labile factors such as trypsin inhibitor and lectins in Jatropha meal and of the heat-stable factors such as antigenic proteins, if any, for common carp. Incorporation of Jatropha kernel meal that was subjected to heat treatment for >15 min decreased growth performance of common carp. These findings imply the loss of amino acids and their lower availability due to Maillard reaction products or heat-induced changes in the struc-

ture of Jatropha proteins, or a combination, which are less digestible by the fish intestinal proteases. The energy retained in the fish was also lower in the 30 and 45 minute-heated Jatropha meal-fed groups compared with the unheated meal fed group. However, heat treatment has been shown to increase protein digestibility of Jatropha protein by rumen proteases (Aderibigbe *et al.*, 1997) and also to inactivate the trypsin inhibitor and lectin (Makkar & Becker, 1997). Nutrient retention in the whole body was similar for control, unheated and heated (15, 30 or 45 min at 121°C) Jatropha meal-fed groups.

Guidelines for using detoxified kernel meal and detoxified protein isolate from Jatropha curcas as a protein source in animal feed

Based on literature studies, the detoxified Jatropha kernel meal (DJPKM), detoxified Jatropha protein isolate (DJPI) and non toxic Jatropha kernel meal (NTJPKM) can replace 50, 62.5 and 75% fishmeal protein, respectively, in fish diets, without sacrificing growth and nutrient utilization, and without affecting physiological and haematological parameters. For shrimp, 50% fishmeal protein could be replaced by DJKM. The guidelines described below would increase the efficiency of DJKM, NTJPKM and DJPI utilization in fish and shrimp.

- Take into account that DJKM and NTJPKM contain approximately 65% crude protein, which is similar to the level in fishmeal, and can therefore substitute for fishmeal on an equal weight basis.
- The acceptability of DJKM, NTJPKM and DJPI-based diets by fish, as measured by immediate consumption and no waste in the tanks, is good.
- DJKM, NTJPKM and DJPI are deficient in lysine. Therefore, lysine monohydrochloride should be supplemented at a level of 1.5% of the DJKM, NT-JPKM and DJPI (w/w) inclusion in the diet to compensate for the deficiency.
- DJKM and NTJPKM contain approximately 9–10% phytate, which is almost 3-fold that in SBM. To mitigate its effect, add 1,500 FTU phytase per kg of diet (Kumar *et al.*, 2011).
- Detoxified Jatropha kernel meal-, NT-JPKM- and DJPI based diets could be fed to fish at 5 times maintenance requirements. Single maintenance requirement equals 3.2 g

feed/kg metabolic body mass (kg 0.8) per day. Shrimp (juveniles, >10 g) should be fed 3-4% of the total body weight per day.

Potential challenges in using detoxified kernel meal and detoxified protein isolate from ***Jatropha curcas*** **in feed**

- Inadequately heated Jatropha kernel meal that contains significant amounts of trypsin inhibitor and lectin could reduce the performance of monogastric animals. Similarly, inadequately detoxified material containing phorbol esters could cause adverse effects. **Phorbol esters must be below the detectable limit (<3µg/g meal).**
- Overheating Jatropha kernel meal could increase the portion of 'bound protein', which is indigestible and denature the protein compositure.
- Incorporating a high level of DJKM into a diet requires rebalancing the ingredients in order to maintain a proper protein energy ratio.
- Fish and shrimp fed high levels of plant protein such as DJKM or DJPI could deposit more fat in their fish muscles, which could be more unsaturated and hence more susceptible to oxidation.

Tests results of use of Jatropha kernel cake in diets of ruminants

Generally, ruminants are considered to be less prone to the effects of toxins compared to monogastrics, due to the presence of microbes in the rumen. For example, rumen microbes can degrade trypsin inhibitor, lectins and phytate to a substantial extent, whereas monogastric animals cannot. Degradation of *J. curcas* phorbol esters by rumen microbes, using an *in vitro* rumen fermentation system, has been investigated by Makkar and Becker, who conclude that rumen microbes do not degrade phorbol esters present in *J. curcas* seeds, and phorbol esters do not adversely affect rumen fermentation. Ruminants would be as susceptible as monogastrics to phorbol ester-mediated toxicity of Jatropha seeds. The few experiments carried out on ruminants confirm the above researches, highlighting the above conclusions:

- ground *J. curcas* seed, given to 6-8-month-old healthy male Nubian goat kids in repeated daily oral doses of 0.25 g/kg of live weight, was lethal;
- oven-dried *J. curcas* kernel cake used in the diet formulation for West African dwarf goats, at levels of 50 and 100% as replacement for soybean cake, determined persistent diarrhoea, poor feed intake, dehydration and death;
- a processed *J. curcas* seed cake with about 220 mg/kg of PE, substituting 12% of crude protein in the diet, led to a reduced nutrient intake and unusual blood metabolite levels in sheep.

A Brazilian experience evaluated the performance of grazing dairy cows supplemented with concentrate containing 0, 10, 20 and 30% of detoxified *Jatropha curcas* L. expeller meal. The inclusion of up to 10% detoxified *Jatropha curcas* L. expeller meal containing about 0.020 mg/g of PE (corresponding to about 10 mg of PE intake/animal/day) does not affect the intake level and milk yield and the inclusion of up 20% of the same detoxified meal doesn't affect the yield of fresh cheese and its chemical and sensory characteristics. Furthermore, it is possible that the Jatropha meal cake contained antinutritional factors (as saponins, trypsin inhibitor, etc.) not inactivated by alkali detoxification process, which may have contributed to the reduction in consumption of supplements from 20% of inclusion detoxified meal cake.

5.2. Jatropha curcas use for goat feeding

The aims of the experiment were the following:

- verify the effect of low levels of phorbol esters (PE) intake on young goat performances (growth rate and feed utilization): trial 1;
- evaluate the nutritive value of *Jatropha curcas* (JC) cake at low levels of PE: trial 2;
- verify the effect of PE consumption on young goat health: trial 3;
- measure the level of PE contamination of the body tissues and organs of young goat consuming JC cake: trial 4.

5.2.1. Jatropha curcas accessions

The seeds utilized to produce the experimental cakes, belonged to two different accessions of Jatropha curcas L.: one from Cautla, State of Morelos, Mexico, (18° 48′ 26.35″ North, 98° 57′ 35.92″ West, 1289 m a.s.l., 890 mm annual rainfall and 16.6°C mean temperature), supposed low-toxic (NJS), because eaten by local people, as indicated by University of Durango, State of Durango, Mexico, which provided the material and the other coming from Prampram, Greater Accra region, Ghana, (5° 43′ 21.46″ North, 0° 05′ 57.29″ East , 11 m a.s.l., 806 mm annual rainfall and 26.6°C mean temperature), supposed toxic (TJS), as indicated by the supplier company. The main seed characteristics of the above accessions are reported in Table 1.

5.2.2. Jatropha curcas seed cakes and concentrates

Different seed cakes were obtained at Experimental Farm of Udine University, Udine, Italy, by processing *J. curcas* seeds with a mechanical single screw press MPS 60 MT (Mailca, Parma, Italy), powered by a 5.0 kW motor, adopting the methodology already described by Baldini et al. (2014). After oil extraction, the following seed cakes were provided: a low toxic cake, obtained through pressing integral NJS; a toxic cake, obtained by pressing integral TJS; and a toxic, partially decorticated cake, obtained by pressing the toxic Jatropha seeds, previously partially decorticated (TDJS; Table 1). In particular, a mechanical dehuller, calibrated to remove about 30% of the shell before seed pressing, was utilized in order to obtain the partially decorticated seed. After that, the supplier company submitted the toxic and the toxic, partially

decorticated cakes to a detoxification process in order to strongly decrease the amount of phorbol esters and allowing its use in the animal feeding trial.
At the end of the above processes, four different experimental *J. curcas* seed cakes were got: low-toxic cake (JN); toxic cake (JT); detoxified cake (JD); detoxified partially dehulled cake (JDD). All the Jatropha seed cakes were oven-dried (130°C x 30 min. at high humidity) in order to inactivate both trypsin inhibitor (TIA), and lectin activities. This treatment was necessary in order not confuse any toxic effects of the above anti-nutritional compounds with those of phorbolesters, which, on the contrary, are not affected by the temperature treatment applied (Gubitz et al., 1997).

Five pelleted concentrates were used for feeding the young goat, a commercial one and four iso-protein and iso-energetic experimental concentrates: JN, JT, JD or JDD. Each experimental concentrate contained 25% of one of the oven-dried Jatropha cakes. The experimental concentrates were produced in the feed laboratory of the University of Udine. All ingredients were ground through a 0.5 mm sieve before final mixing and dry pelleting through a 5 mm dye. The main characteristics of the seeds, commercial and experimental concentrates are reported in Table 1, together with the characteristics of the hay completing the daily ratios of the young goats.

5.2.3. Animals, diets and experimental design

The research was performed in conformity with the Italian regulation concerning animal' protection for experimental purposes (permission granted by Health Ministry, according to the Law Decree no.116/92). It was carried out in Pagnacco (Udine, northeast Italy), in the experimental farm of the University of Udine, on Alpine goat male kids of approx. 3 months of age. During the different phases of the investigation, 20 goats (experimental animals) were fed on diets comprising meadow hay, a commercial and one of the experimental concentrate feeds, while 13 goats (controls) were fed on hay and commercial concentrate feed, at a comparable forage to concentrate ratio. The experimental subjects were kept in individual pens for goats weighing less than 70 kg (Law Decree no.116/92); conversely, the controls were reared on three multiple boxes. Pens and boxes on sawdust litter were furnished of automatic waterers.
Three weeks before the experiment starting, goats were treated for endoparasites.

The study was organized in different trials. The experimental design and the chronological sequence of the different trials is reported in Figure 1.

Experimental group			Pre-treatment (weeks)				treatment (weeks)				
Name	**Treatment**	**Goat (no.)**	**1-3**	**4-6**	**7-9**	**10-12**	**1-3**	**4-6**	**7-9**	**10-12**	**13-15**
t1	treated	6									
t2	treated	6									
t3	treated	2									
t4	treated	6									
C0	control	13									

Trials:

Pre-treatment period of growth rate recording and no-treatment period of control group

PE intake effect on goat performance (1)

Nutritive value of detox and low-tox JC (2) and PE effect on animal health (3 and 4)

PE effect on animal health (3 and 4)

Figure 1. Experimental design and trial time-sequence (see the text for detailed description of the trials).

5.3. Effect of low levels of PE intake on young goat performances (trial 1)

The JT concentrate, made with 25% of JT cake, was provided to 20 Alpine goat male kids, at growing levels and for three periods of different length (3, 6 or 12 weeks), preceded by a pre-treatment period. The performances of these goats were compared with those of a control group comprising coetaneous goats belonging to the experimental farm flock.

Table 1. Proximate composition of concentrates, Jatropha cakes and hay.

	DM (%)	ASH (DM%)	CP (DM%)	EE (DM%)	NDF (DM%)	NE[1] (MJ/kgDM)	PE (ppm)
Seeds:							
NJS	93.0	5.0	18.0	37.0	42.5	--	20
TJS	94.0	4.5	16.5	27.5	46.5	--	2580
TDJS	94.0	4.5	19.0	31.5	39.5	--	2490
Concentrate:							
Commercial	89.3	9.7	17.7	3.9	27.1	7.15	--
With 25% of Jatropha cake							
JT	87.3	6.0	17.9	5.5	26.0	7.68	105
JN	87.5	6.8	17.5	7.3	26.1	7.75	8
JD	87.2	8.5	17.5	5.2	29.0	7.28	14
JDD	87.5	8.8	17.5	6.8	24.6	7.57	7
Meadow hay:							
	91.3	8.5	8.7	2.3	64.6	--	--

[1] NE = net energy for lactation calculated according to INRA standard.
DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fibre; PE = phorbol esters, expressed as phorbol-12-myristate 13-acetate equivalent in the kernel.
JT = from toxic seeds; JN = from non-toxic seeds; JD = detoxified; JDD = detoxified from

The animals were weighed every two weeks for evaluating their growth rate by regressing the live weight (LW) on the time, during two consecutive periods, before and during the treatment. In Table 2 is reported the experimental design, describing the number of animals involved in each group, the length of the periods and the average LW.

The supplied feeds and feed refusals were individually recorded every day. At the end of the trial, the faeces and urines were daily and individually collected for a four-day period, in order to estimate the diet digestibility. The presentation of the experimental design is completed in Table 3, where the *Jatropha curcas* supplementation and intake are detailed group by group.

The goats supplied with the highest levels of JT concentrate (200 g/day), consumed only a small

amount of the feedstuff that can be considered a voluntary intake estimate, due to the large quantity of refusals left in the trough. The only one goat which consumed 200g/day of JT concentrate for two consecutive days, entered a one-week period characterized by persistent diarrhoea and very poor feed intake. The animal then recovered its apparent normal health condition during the following week.

The highest levels of JT concentrate consumption allowed a phorbol esters intake very lower than the estimated LD50 for mice (7 to $9*10^{-3}$ LD50). The levels of PE intake of the treated goats ranged from 0.5 to 6.3 mg/kg of total dry matter (DM) intake, corresponding to 0.02 – 0.24 mg/kg LW.

The growth performance is reported in Table 4. The JT intake decreased the goat growth. However, the reduction was significantly different from that faced, as a consequence of the normal seasonal path of growing, by the control group only for the two groups fed on the higher levels of JT. The last one, in particular, underwent a negative period of growth, likely due to both, the PE consumption and the reduction of intake, to avoid the ingestion of the concentrate containing the toxic cake.

It is interesting to note, that all the treated goats gradually recovered their growth rate. Indeed there was a low (6.5-8.3 g/week of growth rate), but significant and favourable effect of the length of the treatment on the animal performance.

At the end of the growth period, the goat digestibility was evaluated and expressed as Organic Matter digestibility coefficient (OMd; Table 4). Within the low levels of PE exposition reached in the experiment, due to the ingestion of small amounts of *J. curcas* cake from toxic seeds, the young goat digestibility was not significantly affect (Table 5). Moreover, it was observed a linear increase of the OMd as the length of the treatment period before the digestibility assessment increased (0.52% a week). These outcomes could be a consequence of a progressive ad- aptation of the goat to a chronic, low level of JT intake or this adaptation could include even a slightly reduction of Jatropha intake. Indeed as reported in Table 2 the level of JT concentrate intake decreased along the treatment period likely due to the well-known selective feeding behaviour of goats.

Table 2. Experimental design, describing the average length of the growth period and the goat weight.

	Treatment		Growth period (day)			Live weight (kg)		
Group	Goat (no.)	JT concentrate supply (g/day)	before treatment	treatment	total	initial	at the start of the treatment	final
Control[1]	13	0	80	39	119[b]	11.1	21.8	25.7[b]
Treated	4	6	85	63	148[a]	11.2	21.9	28.3[ab]
	6	12	87	49	136[ab]	11.1	23.8	28.8[a]
	6	55	94	49	143[a]	11.5	22.5	26.4[ab]
	4	200	93	31	124[b]	11.1	24.0	24.6[b]
mean			88	46	134	11.2	22.8	26.7
SE			3.2	3,7	3.1	0.39	0.69	0.37

[1]: for the control goats, the 'treatment' data were recorded during the period of treatment of the treated group.
[a,b,c]: means on the column with different superscript are different, $P<0.05$ (Bonferroni test).
JT = from toxic seeds.

Table 3. Experimental design, describing the JT supplementation, and intake actually obtained.

Treatment				Daily intake of *Jatropha curcas*					
Group	Goat (no.)	JT concentrate supply (g/day)	Length (weeks)	Concentrate (g of DM)	Cake (g)	PE (mg)	PE (mg/kg DM intake)	PE (mg/kg LW)	PE[1] (‰ LD_{50})
Control	4	0	--	0.0	0.0	0.0	0.0	0.00	0.0
Treated	2	6	6	4.0	1.1	0.5	0.5	0.018	0.65
	2		12	4.9	1.4	0.6	0.6	0.023	0.84
	2	12	3	11.3	3.2	1.4	1.2	0.048	1.7
	2		6	11.3	3.2	1.4	1.4	0.052	1.9
	2		12	9.9	2.8	1.3	1.3	0.047	1.7
	2	55	3	48.8	13.7	6.2	6.0	0.236	8.6
	2		6	45.0	12.6	5.7	5.9	0.217	8.0
	2		12	38.4	10.8	4.8	4.2	0.205	7.5
	2	200	3	37.6	10.6	4.8	6.3	0.194	7.1
	2		6	30.8	8.6	3.9	5.2	0.167	6.1

[1]: LD_{50} = 27.34 mg PE/kg LW.
JT = from toxic seeds; PE = phorbol esters.

Table 4. Influence of PE ingestion on growth rate of young goat.

Treatment			Average daily gain (g/day)			
Group	Goat (no.)	JT concentrate supply (g/day)	before treatment	during treatment	difference	total
Covariate model						
Control[1]	13	0	130	124[a]	6[b]	128
Treated	4	6	131	110[ab]	21[ab]	122
	6	12	139	85[ab]	54[ab]	126
	6	55	113	63[bc]	50[ab]	100
	4	200	150	-21[c]	171[a]	108
mean			133	70	63**	117
SE			6.3	6.8	9.9	4.9
Effect of the length of the treatment			n.s.	**	**	n.s.
Regression model			n.s.			
Intercept		coefficient		77.1**	66.6*	128**
		SE		17.90	26.68	5.33
PE daily intake		coefficient		-16.5**	15.8**	-4.4**
(mg PE /kg LW)		SE		2.71	4.04	1.68
Length of the treatment (week)		coefficient		6.54**	-8.30*	n.s.
		SE		2.344	3.493	

[1]: for the control goats, the 'treatment' data were recorded during the period of treatment of the treated group.
[a,b,c]: means on the column with different superscript are different, P<0.05 (Bonferroni test). [**]: *P*<0.01; [*]: *P*<0.05; n.s. = not significant. JT = from toxic seeds; PE = phorbol esters.

Table 5. Coefficient of organic matter apparent digestibility (OMd, %) and its sources of variability.

Group	Goat (no.)	OMd (%)		Concentrate level (% DM intake)[1]		Treatment length (week)[2]		Treatment intensity (PE mg/kg LW)
		mean	SE	mean	SD	mean	SD	
Control	4	69.4	1.06	0.37**	0.089	--	--	--
Treated	20	71.8	0.58			0.52**	0.159	n.s.

[1]: average concentrate level at which the average value of 71.8% was estimated = 66.6% of the ingested DM.
[2]: treatment length at which the average value of 71.8% was estimated = 6 weeks. **: $P<0.01$; n.s. = not significant.
PE = phorbol esters.

5.4. Nutritive value of cake of Jatropha curcas at low levels of PE (trial 2)

Twelve goats, coming from the previous trial, divided in three experimental groups of four animals each, were fed on the already described JN, JD and JDD concentrates, in partial substitution of the commercial one, in order to keep the levels of PE ingestion around 2-4 mg/kg DM intake (>5‰ LD_{50}, where LD_{50} = 27.34 mg PE/kg LW). The diet was completed by a meadow hay (25% of the consumed diet). Every treatment lasted 3 or 6 weeks, on two goat each. Four control goats, receiving the commercial concentrate and the meadow hay (75% to 25% ratio), were also included in the trial.

The supplied feeds and feed refusals were individually recorded every day. At the end of the experimental period, the faeces and urines were daily and individually collected for a four-day period, in order to estimate the diet digestibility. The presentation of the experimental design is completed in Table 6, where the *Jatropha curcas* supplementation and intake are detailed group by group.

Table 6. Experimental design and intake of the digestibility trial.

	Control	JN	JD	JDD	SE
Dry matter intake (DMi; g/kg $LW^{0.75}$)	74	72	71	73	0.66
Total concentrate (%)	74.5	74.1	74.1	74.5	0.06
Commercial concentrate (g/day)	810	500	500	250	--
JC concentrate (g/day)	0	290	290	580	--
JC cake (g/day)	0	72.5	72.5	145	--
PE (mg/kgDMi)	0	2.4	4.0	4.2	0.01
PE[1] (‰ LD_{50})	0	2.7	4.4	4.7	0.00

[1]: LD_{50} = 27.34 mg PE/kg LW.

JC = *Jatropha curcas*; JN = concentrate from non-toxic seeds; JD = concentrate detoxified; JDD = concentrate detoxified from decorticated seeds; PE = phorbol esters.

The main results of digestibility trial are reported in Table 7.

Table 7. *In vivo* digestibility of nutrients (apparent digestibility coefficient, d, %).

	Control	JN	JD	JDD	SE
OMd	69.7[a]	65.6[b]	64.3[b]	69.6[a]	0.42
CPd	74.4[a]	69.9[b]	66.9[b]	68.6[b]	0.62
NDFd	43.0[a]	38.6[b]	37.4[b]	42.8[a]	0.58

[a,b]: means on the rows with different superscript are different, P<0.05 (Bonferroni test).
OMd = organic matter; CP = crude protein; NDF = neutral detergent fiber.
JN = concentrate from non-toxic seeds; JD = concentrate detoxified; JDD = concentrate detoxified from decorticated seeds.

Within a maximum intake of 580 g/day of JC concentrate (corresponding to 145 g/day JC cake), the variations of the nutritive value of the diet after the inclusion of Jatropha cake are due to the husk presence, that significantly reduces the digestibility. Indeed, as shown in Table 7, the coefficient of organic matter digestibility (OMd), expression of the nutritive value of the diet, did not change by including in the control diet 15% of JDD cake. By contrast, the presence of 7% of not decorticated cake, from both non-toxic (JN with 7.5 ppb PE) or detoxified (JD with 14.5 ppb PE) seeds, significantly reduced the OMd. This result is influenced by the fibre digestibility, expressed in term of digestibility of Neutral Detergent Fiber (NDFd) that decreased of approx. 10% in the diet containing the seed husk.

The Jatropha cake inclusion significantly diminished the digestive utilization of the nitrogen components of the diet (from 6 to 10%), independently of the cake origin and quantity. The Crude Protein (CP) provided by Jatropha ranged from 9 to 16% of total CP of the experimental diets. The heating treatment for inactivating the potential anti-nutritional factors of the cake, other than PE, likely contributed to that phenomenon, reducing the Jatropha protein availability.

Conclusions

At the tested levels of PE contamination (30-50 ppm in the cake) and cake inclusion in ruminant diets (7-15%), corresponding to 2-4.5 ppm in the diet, the nutritive value of the by-product is not affected by the PE presence. By contrast, the nutritive value is strictly linked to the technological processing adopted to make the Jatropha cake. Among the others, particularly important are the presence of the husk and the procedures to inactivate its antinutritional factors (temperature level and time).

5.5. Effect of PE consumption on young goat health (trial 3)

The 20 experimental kids were submitted to a controlled period of PE assumption, by both concentrate intake or cake dosage, after which they were slaughtered for anatomopathological evaluation and tissues sampling for histological analysis. The intoxication treatment is summarised in Table 8. During the treatment, the animals' health was daily assessed by an experienced veterinarian, using a checking list of toxicity symptoms sorted by increasing level of severity, from lack of appetite to severe diarrhoea.

Only three goats, those submitted to the highest level of intoxication, showed some clinical symptoms. The animal ID 3 (Table 8), after 5 days of treatment was affected by respiratory disease, and then the signs of this illness confused the potential ones derived from a possible intoxication. The treatment of this animal was suspended at day 10.

The animal ID 1 presented the most severe toxicity symptoms: lack of appetite from 2nd day; ataxia from the 8th day; cachexia from the 12th day. The animal ID 2 showed only a lack of appetite from 8th day.

Table 8. PE intoxication treatment of young male goats before slaughtering average LW 30 kg).

Goat ID	JC cake	Treatment			Administration		
		type	days of admin-istration	days of sus-pension	JC g/d	PE in JC mg/kg	PE mg/d
1	JT	dosed	15	0	83.0	420.0	34.9
2	JT	dosed	15	0	83.0	420.0	34.9
3	JT	dosed	10	5	26.8	420.0	(11.3)
4	JT	dosed	15	0	26.8	420.0	11.3
5	JT	dosed	15	0	20.2	420.0	8.5
6	JT	dosed	15	0	20.2	420.0	8.5
7	JT	dosed	15	0	13.5	420.0	5.7
8	JT	dosed	15	0	13.5	420.0	5.7
9	JDD	ingested	21	0	145.0	28.7	4.2
10	JDD	ingested	21	0	145.0	28.7	4.2
11	JDD	ingested	42	0	145.0	28.7	4.2
12	JDD	ingested	42	0	145.0	28.7	4.2
13	JD	ingested	21	0	72.5	52.0	3.8
14	JD	ingested	21	0	72.5	52.0	3.8
15	JD	ingested	42	0	72.5	52.0	3.8
16	JD	ingested	42	0	72.5	52.0	3.8
17	JN	ingested	42	0	72.5	32.1	2.3
18	JN	ingested	42	0	72.5	32.1	2.3
19	JN	ingested	21	0	72.5	32.1	2.3
20	JN	ingested	21	0	72.5	32.1	2.3
21	--	Control	.	.	0	0	0
22	--	Control	.	.	0	0	0
23	--	Control	.	.	0	0	0
24	--	Control	.	.	0	0	0

JC = *Jatropha curcas*; PE = phorbol esters; JT = from toxic seeds; JDD = detoxified from decorticated seeds; JD = detoxified; JN = from non-toxic seeds.

Anatomophatological evaluation and histological analysis of the organs of young goats exposed to increasing levels of PE from Jatropha curcas cake

The scheme of anatomopathological evaluation and histological analysis is summarized in Table 9 while the results are reported in detail in Table 10.

Table 9. Animals submitted to the anatomopathological evaluation and histological analysis.

Goat ID	PE ingested (PE mg/day)	ANATOMOPATHOLOGICAL EVALUATION	HISTOLOGICAL ANALYSIS
24	Negative check	DONE	DONE
22	Negative check	DONE	DONE
21	Negative check	DONE	DONE
17	2.3	DONE	DONE
15	3.8	DONE	DONE
13	3.8	DONE	DONE
10	4.2	DONE	DONE
8	5.7	DONE	DONE
6	8.5	DONE	DONE
4	11.3	DONE	DONE
2	34.9	DONE	DONE
1	34.9	DONE	DONE

PE = phorbol ester.

Table 10. Descriptive anatomopathological evaluation and histopatological analysis.

Goat ID	PE ingested (mg/day)	ANATOMOPATHOLOGICAL EVALUATION	HISTOPATHOLOGICAL ANALYSIS
24	Negative check	No abnormality in the internal organs (lungs, heart, liver, spleen, forestomachs, gland stomach, pancreas, intestine).	Subject's organs have no abnormality. A modest congestion caused by slaughter practices.
22	Negative check	No abnormality in the internal organs (lungs, heart, liver, spleen, forestomachs, gland stomach, pancreas, intestine).	Subject's organs have no abnormality. A modest congestion caused by slaughter practices.
21	Negative check	No abnormality in the internal organs (lungs, heart, liver, spleen, forestomachs, gland stomach, pancreas, intestine).	Subject's organs have no abnormality. A modest congestion caused by slaughter practices.
17	2.3	No abnormality in the internal organs (lungs, heart, liver, spleen, forestomachs, gland stomach, pancreas, intestine).	Subject's organs have no abnormality. Slight entity in the liver, but attributed to a normal situation. A modest congestion caused by slaughter practices.
15	3.8	No abnormality in the internal organs (lungs, heart, liver, spleen, forestomachs, gland stomach, pancreas, intestine).	Subject's organs have no abnormality.
13	3.8	No abnormality in the internal organs (lungs, heart, liver, spleen, forestomachs, gland stomach, pancreas, intestine). However, **abomasum** and **duodenum** have a slightly congested mucosa.	Subject's organs have no abnormality, apart from a modest congestion in the **duodenum**.
10	4.2	No abnormality in the internal organs (lungs, heart, liver, spleen, forestomachs, gland stomach, pancreas, intestine).	Subject's organs have no abnormality.
8	5.7	Internal organs have no abnormality, except **duodenum**, which has a slightly thickened and congested mucosa.	Subject's organs have no abnormality, but there is a clear congestion in **duodenum** walls, with slight edema.

6	8.5	Subject's organs has no abnormality, except **intestinal tract**, especially **duodenum,** which has a slightly thickened and congested mucosa.	**Intestine**: increase the loss of mucosa's surface layers and remarkable congestion of the wall. **Liver**: hepatocytes with rarefied and light cytoplasm (steatosis); congestion of portals and centrilobular veins. Plasma stagnation inside these last ones. **Pancreas**: reduction in the number and dimension of Islets of Langerhans.
4	11.3 No Suspension	Subject's organs has no abnormality, except **intestinal tract,** especially **duodenum** and **abomasum** with a congested mucosa. Ruminal contents appear dark, as well as in the intestine.	**Intestine**: Modest loss of mucosa's surface layers and congestion of the wall. **Liver**: hepatocytes with rarefied and light cytoplasm (steatosis). **Kidney**: widespread congestion and slight bleeding found in glomeruli.
2	34.9	**Liver**: No abnormality. **Kidney**: widely hyperemic. **Intestine**: hyperemic edematous intestinal mucosa, with petechial haemorrhages. **Forestomachs**: rumen, hyperemic mucosa; omasum, mucosa full of surface haemorrhages areas. **Abomasum**: mucosa strongly hyperemic. Lung: hyperemia with petechial haemorrhages.	**Liver**: loss of normal structure of liver cells; cytoplasmic margins undefined; reduction of cell content; lipid infarction of hepatocytes (steatosis); presence of lipofuscin; reduction of nuclear chromatin, congestion of sinusoids. **Pancreas**: reduction of Islets of Langerhans (number and dimensions). **Kidney**: haemorrhages found in the glomerule; vacuolar degeneration in the renal tubules; **Intestine**: strong lymphocyte infiltrate in the lamina propria of some intestinal tracts; congestion of lamina propria capillaries. **Rumen**: reduction of layer thickness of squamous-keratinized-and-stratified epithelium; presence of vacuolated and necrotic cells; in some areas loss and detachment of surface layers of squamous-keratinized-and-stratified epithelium. Lung: congestion of alveolar capillaries; wide haemorrhages.
1	34.9	**Liver**: No abnormality. **Kidney**: widely hyperemic. **Intestine**: wide tracts of intestinal mucosa are strongly hyperemic. **Forestomachs**: rumen, standard mucosa, except some haemorrhagic villi; omasum, mucosa full of surface haemorrhages areas. **Abomasum**: mucosa strongly hyperemic. Lung: hyperemia.	

The three control subjects (24, 22, 21) have no alterations macro- and histologically. Subjects, fed or dosed with low amounts of *Jatropha curcas* cakes (17, 15, 13, 10, 8), have healthy internal organs, from an anatomo- and histopathological point of view, apart from the subject 8 that has the duodenum with congested mucosa. More specifically, the subjects fed with detoxified or low toxic *Jatropha curcas* cake (17, 15, 13 and 10) have no internal organs alteration.

The goats dosed with increasing amounts of toxic *Jatropa curcas* (6 and 4) show a slight but progressive increase of lesions in relation to the increase of active principle concentration. To sum up, gross lesions are in the digestive tract with congestive/hyperemic phenomena (especially in the duodenum, partly in the abomasum). There are hepatocytes with light and small cytoplasm (steatosis) and a congestion of centrilobular veins in the liver. Alterations in the kidney of one subject: wide congestion and slight haemorrhages in the glomeruli.

Regarding the two goats, dosed with the highest levels of Jatropha cake (2 and 1), we have the most significant alterations, characterised by a worsening in the intestinal tract. **Anatomopathologically**, both subjects have forestomachs full of haemorrhagic villi or hyperemic mucosa or surface haemorrhagic areas. Abomasum, which has marker alterations, has a strongly hyperemic mucosa. Intestine has tracts of mucosa, is strongly hyperemic, edematous, with petechial haemorrhages. Kidney appears widely hyperemic, whereas lung, with a slight hyperemia, has small haemorrhagic areas. **Histopathologically**, Intestine is affected by a strong lymphocytary infiltrate in the lamina propria; while the congestion of lamina propria capillaries is widespread. Rumen marks a reduction of layer thickness of squamous-keratinized-and-stratified epithelium, with vacuolised and necrotic cells; in some areas there is the loss and detachment of surface layers of squamous-keratinized-and-stratified epithelium, showing a degenerative suffering caused by prolonged active principle feed. Pancreas has a reduction of number and dimension of the Islets of Langerhans. Then, liver histopathologically shows a loss of hepatic cells structure (muralium), whereas cytoplasmic margins are indefined. Cell content appears rarefied, marking a discrete lipid infarction of hepatocytes (steatosis), with an increase of lipofuscins. In the kidney haemorrhages in glomerulus have worsened, although there is a vacuolar degeneration in renal tubules. Lung shows a wide congestion of alveolar capillaries, and in one of the two subjects there are slight haemorrhages.

These observations disagree with experimental studies by Rakshit *et al*. (2008) on rats, and by Wang *et al*. (2011) on pigs, both fed with different *Jatropha curcas* amounts, which didn't find any alteration micro and macroscopically. Our considerations, indeed, agree with studies

by Adam *et al*. (1974) on mice, marking histological alterations; and totally agree with Cai-Yan Li *et al*. (2010) who worked with different *Jatropha curcas* amounts analyzing liver, steatosis and congestion, lung haemorrhages. Whereas in kidneys, there are glomerular sclerosis and glomer- ular atrophy, probably caused by the different metabolism of these two species.

Katole *et al*. (2011), finally, studied sheep fed with different *Jatropha curcas* amounts, whose anatomical-functional features are similar to those of goats. In this study histo- and anatomo-pathological investigations were not carried out. It is interesting to note that this research marked a significant increase of LDH and SGOT serum in animals, which swallowed higher doses of *Jatropa curcas*. LDH and SGOT are two enzymes, in case of significant increase in the serum, that often show liver diseases, hepatic and/or renal disorders, perfectly similar to our anatomo/histopathological patterns.

Conclusions

The subjects fed with low doses of *Jatropha curcas* (2.3-4.2 mg of PE per day, coinciding with the same value expressed in ppm of diet, since the feed intake was approximatively 1 kg per day) cakes from a low toxicity/detoxified product, have no macroscopically alteration or microscopically.

On the other hand, with regard to animals fed with toxic Jatropha, with increased doses from 5.7 to 34.9 mg of PE per day, we have these results: subjects fed with increasing doses between 5.7-11.3 ppm of PE in the diet, show a correspondence slight increase in lesions in the digestive tract; anyway lesions remain very confined. Whereas goats with the highest dose of Jatropha (34.9 ppm of PE in the diet), have especially evident alterations in the digestive tract.

Our investigation, even though preliminary, leads to the conclusion that with levels of toxic Jatropha ingestion since 6 mg of PE per day (the same 6 ppm in the diet) we have progressive alterations in the digestive system, although moderate, in particular at 6 mg only the duodenum has a congested and thickened mucosa anatomopathologically, with a slight edema histopathologically. Instead, subjects fed with low toxicity/detoxified Jatropha, until levels of 4 mg of PE per day, do not evidence any significant gross and small lesions.

5.6. Level of PE contamination of body tissues and organs of young goats consuming Jatropha curcas cake (trial 4)

On the same animals described above (Table 8), after slaughtering the following organs and tissue were sampled, freeze-dried and stored to be analysed for their PE content: duodenum, reticulum, kidney, liver, kidney fat, *longissimus dorsi* muscle. The day before slaughtering, a blood sample and the whole day urine sample were also collected.

The first results were already published by Baldini *et al*. (2014). In particular the liver of two goats, one control (ID 21) not submitted to any treatment and the other dosed daily with 83 g of a *J. curcas* cake containing 420 mg/kg PE, corresponding to a daily intake of 34.9 mg PE (ID 1) were sampled (100 g of fresh tissue), freeze-dried, ground and then stored at −20°C until the PEs analysis.

HPLC analysis was performed using a Shimadzu (Germany) Prominence XR HPLC system equipped with a diode array detector mod. SPDM20A and a Lab. Solution LC single/PDA data acquisition and processing system. The separation was performed on reverse-phase 4.6 × 150 mm, 5 m C18 Zorbax Eclipse Plus end-capped column (Agilent Technologies, Santa Clara, USA), thermostated at 25°C. The elution was in isocratic mode using a mixture of ACN and water in the ratio of 80:20 (v/v) as mobile phase at a flow rate of 1 mL/min. The sample injection volume was 20 micro L. The detector wavelength was set at 280 nm. Under these conditions, the PE retention time was in the range 8.0–12.3 min. The areas of the chromatographic peaks were used for the quantitative analysis with TPA as external standard. The 12-O-Tetradeca-noylphorbol-13-acetate (TPA) retention time was 19–20 min. The PE concentration in the samples was expressed as TPA equivalent.

The LC–MS/MS analysis was performed using an Agilent UHPLC 1290 Infinity coupled with an Agilent 6490 triple-quadrupole with Ion Funnel (IF) Technology via Jet Stream Ionization (JSI) source (Agilent Technologies).The analytes were eluted, at a flow rate of 0.5 mL/min, from a Zorbax Eclipse Plus C18 column (2.1 × 100 mm–1.8 m) equipped with a C18 guard column and thermostated at 50°C, with a solution mixture (A: water with ammonium formate 2 mM and formic acid [0.01%]; B: CAN). Mobile phase composition changed linearly, after 1 min, from A/B 60/40 to A/B 5/95 in 25 min and hold for 2 min and then re-equilibration at A/B 60/40 for 3.5 min. Agilent JSI source parameters were firstly optimized on TPA by syringe infusion technique and then fine-tuned on the PEs profile. The optimized source under positive

ion mode, ESI (+) conditions were: gas temperature 120°C, gas flow 19 L/min, nebulizer 50 psi, capillary 4500 V, charging 400 V. Triple quadrupole conditions: Ion Funnel 170/80.MRM transitions for PE were the following: 695 → 311 at CE 10; 677 → 311 at CE 15; 293 → 265 at CE 10. TPA (I.S.) was monitored at 600 → 311 with CE 10.

In order to limit matrix effect and improve accuracy determination of PE in complex matrices such as liver samples, quantitation was conducted with the standard addition method. After preliminary run analysis for estimating the presence of PE in the liver extracts, each sample was added with a known amount of PEs, 10 and 20 micro g/g, and initial PEs concentration was calculated from the curve by means of extra No PE-related peaks were found in either liver extract, regard-less of whether the animal had been fed with PEs or not although the treated animal presented clear histopathological symptoms linked to the effect of PE (Table 10). The results of this experiment, which represents a preliminary approach, can be explained by the fact that xenobiotics, especially lipophile molecules, undergo processes of hepatic biotransformation with the formation of metabolites not identifiable under these experimental conditions.

5.7. References

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CHAPTER SIX

LIMITATIONS, GENERAL CONCLUSIONS, AND PERSPECTIVES

6.1 Limitations of the Study

The precisions of the outcome of the studies reported in this dissertation reflect the practical realities of production under extensive ranching conditions of the Western highland plateau savannah of central Africa. The prevailing weakness in the animal healthcare delivery sytem and inefficient and or absence of relevant policy regulating cattle health and production probably impacts on the the expressed obseravtions made on the experimental model used in assessing the body measures and carcass characteristics of the two genotypes investigated. The length of time required for development of the second filial generation of the experimental animals *vis a vis* time and paucity of funds available circumscribed our observations soley on the first filial generation of the young bulls destined for beef production.

6.2 General Conclusions

The potentials of the use of *Jatropha curcas* as a novel protein feed integrator for ruminant nutrition; at the tested levels of phorbol ester (PE) contamination (30-50 ppm in the cake) and cake inclusion in ruminant diets (7-15%), corresponding to 2-4.5 ppm in the diet, the nutritive value of the by-product is not affected by the PE presence. By contrast, the nutritive value is strictly linked to the technological processing method adopted to produce the jatropha cake. Among the others, particularly important are the presence of the husk and the procedures to inactivate its anti-nutritive factors (temperature level and time). Our investigation on the prevalence and disease resistance marker alleles showed that clinical dermatophilosis is common among zebu Goudali (G) and Simgoud (SG) cattle in the western highland plateau savannah of Cameroon and that resistance cannot be predicted solely on the variability of BoLA-DRB3 gene. With respect to *in-vivo* performance of authocthonouse Goudali and thier crosses with the Italian Simmental we obseved that G pure breed has much lower *in vivo* and at slaughter performance than their crosses with Simmental breed (SG). This is probably because of the additive and heterosis effects that interact with the suboptimal environmental conditions of the genotype and underlines the need to optimise crossbreeding effects by improvement of transfer and pre-slaughter conditions

The study within a broader whole supply value chain approach, validates the relationship between lysine-rich low-toxic *Jatropha curcas* cake, environmental adaptability; with respect to

endemic dermatophilosis and livestock productivity improvement. This, considering the lowering effect on sector environmental footprints; increasing its feedstock flexibility and security while improving the social acceptance of both Jatropha cultivation and intensive animal breeding and invoking the need for an increase in the level of animal welfare along the beef production chain in Central Africa

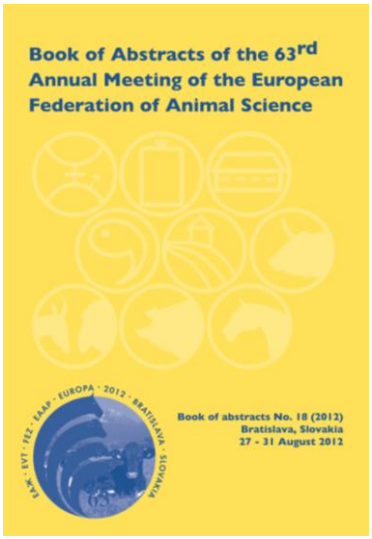
6.3 Study Perspectives

In the near future, improving beef production under low input farming systems in Central Africa would probably not only rely on pasture availability but on the inherent potential of cattle to produce under such limitations. The development of alternative feed sources that impacts positively on the carbon handprints and which do not affect food crop production destined for direct human consumption is imperative. Strategic breeding options for sustaining a positive and progressive increase in productivity of cattle as well as improving on fitness characteristics to cope with the production environment are essential determinants for sector development.

Beyond individual heterosis of F1 observed by body measures and carcass traits and considering the expected positive influence of maternal heterosis from the first filial generation of the SimGoud crops it is inviting for studies related to maternal traits such as calving percentages, weaning weights, longevity in the dam, and other reproductive traits to be conducted. This is even more so as enhanced production from the crossbred female is the primary benefit from a planned crossbreeding system. Therefore, it makes sense to seek information on the crossing of straightbred bull on crossbred females to take advantage of maternal heterosis. Such a study within the context of ranching may be useful to contributing new information to the body of knowledge related to this subject under this specific context and provide industry with pre-requisite information needed for planning and decision-making during production.

7. Annexes

7.1 Annex 1- Presentation at the 63rd EAAP Congress, Bratislava 2012



Session 04a Poster 10

Cattle systems in Misaje area, Cameroon: biomass resource pressure and decentralisation challenges

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Misaje Subdivision is a Sudan savannah grassland of Cameroon at its Northwest frontiers with Nigeria (lat. 6°59' long. 10°55') and an important hotspot for beef-type cattle production; providing livelihood for over 70% of the population. The aim of the study was to audit the prevailing cattle farming systems and profile the challenges on their sustainability. Data set for analysis was obtained from field visits and a questionnaire survey conducted in early 2012. Of 164 farmers surveyed, who managed 213 herds with 17,000 cattle, 88% are landless and predominantly of the minority Mbororo cultural decent, while 12% are indigenous farmers including a 38,000 hectare ranch, breeding 6,200 Goudali cattle, owned by a parastatal; SODEPA. Except for SODEPA ranch, transhumance is the main pastoral system. Three main zebu breeds were identified in transhumant herds. One hundred and forty one herds were of homogeneous breeds (Goudali 27.2%, Aku 22.1%, Djafun 16.9%) while 72 herds where of mixed breeds. Minimum, mean and maximum herd size were 23, 79 and 270 respectively, while most of the herds (55%) fell within 40-80 class size. Shortage of pasture, agro-pastoral conflicts, cattle rustling, and reduced fertility were the determinants for migration during the dry season. Cultivation of cereals; notably maize, was intended for domestic use. Apart from micro-mineral licks, feed supplementation with farm residues is not practiced. The only pasture maintenance action was off-season bush fires. However, a combination of natural pasture rotation and bush fires was noted in SODEPA ranch. Access to land by the Mbororo cattle-rearing minority, in a situation where natural resource management is decentralised, may not be in favour of production. In the absence of specialised seed-stock breeders and organised pasture development and maintenance plans, the prevailing situation is chaotic and unsustainable; lacking essential elements for management and genetic progress.

7.2 Annex 2- Body measurements photo report

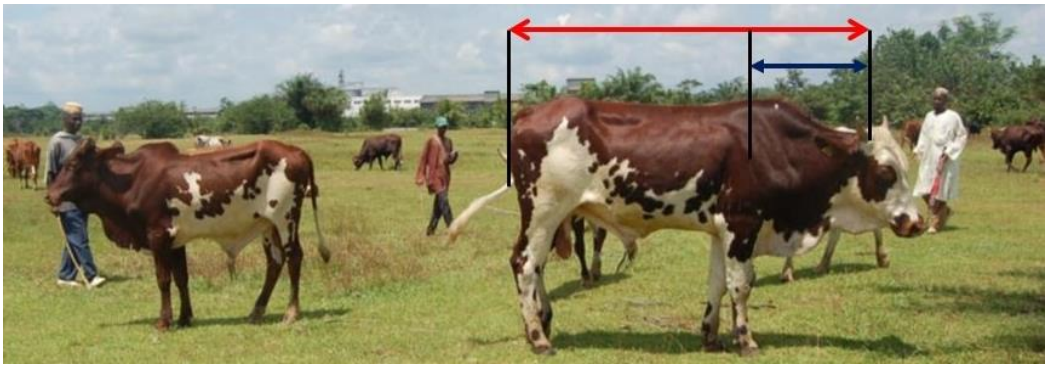

Figure 1. The total top line (red line) and neck length (blue line).

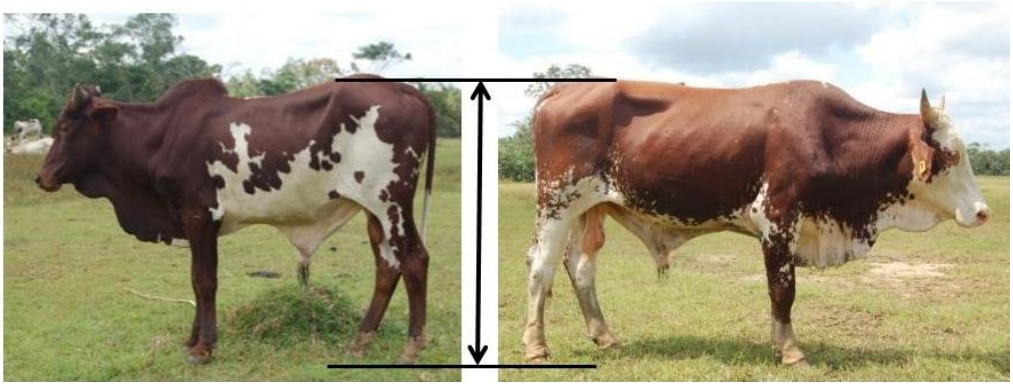

Figure 2. The hip height.

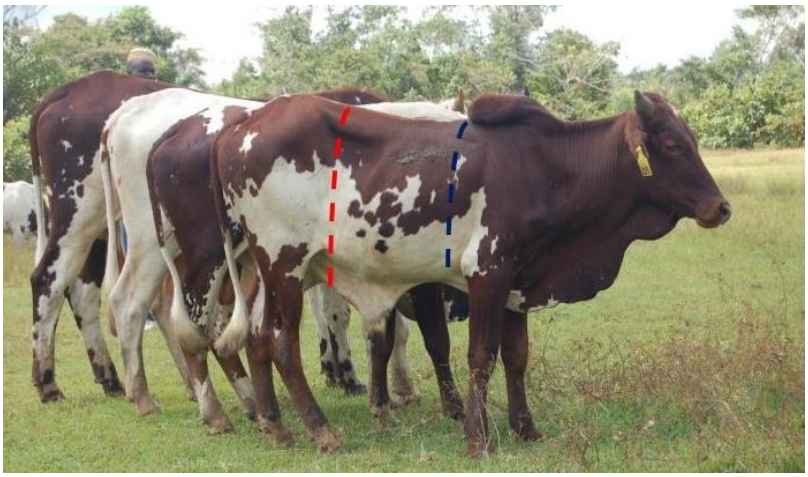

Figure 3. The thoracic circumference or heart girth (blu line) and the flank circumference (red line).

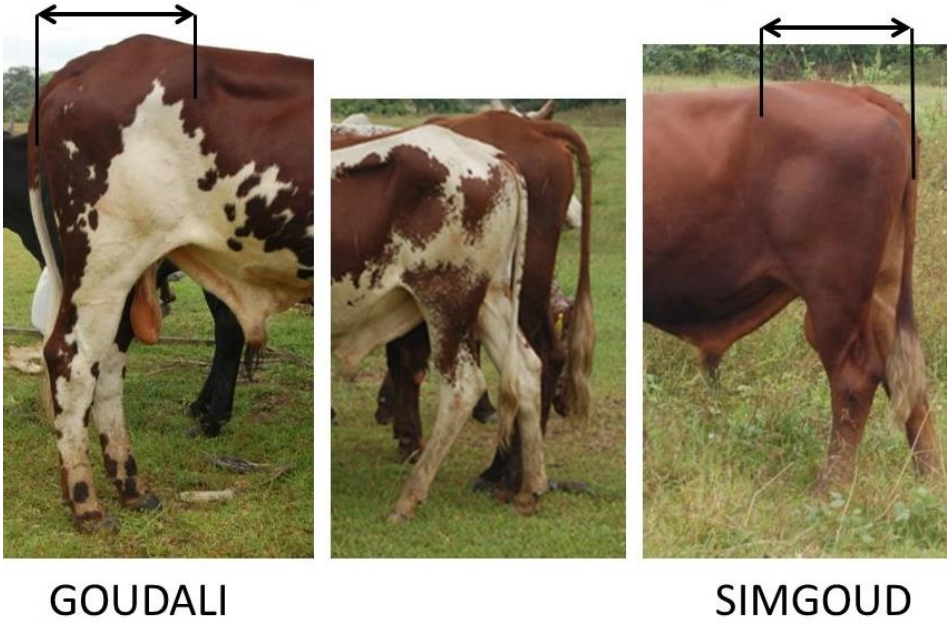

Figure 4. Length and shape of rump in Goudali and SimGoud bulls.

Figure 5. The rump width.

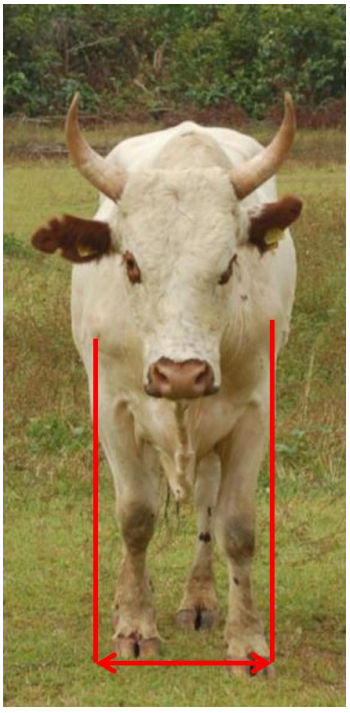

Figure 6. The shoulder width.

Fig. 7. The chest depth.

7.3 Annex 3- Contribution at the 61st ICOMST Congress, Clermont-Ferrand, 2015.

CARCASS AND MEAT CHARACTERISTICS OF YOUNG BULLS FROM LOCAL GOUDALI (ZEBU) CATTLE OF CAMEROON AND ITS CROSSES WITH ITALIAN SIMMENTAL

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Abstract – The objective of this study was to compare *in vivo* and at slaughter performances and *Longissimus thoracis* (LT) composition of Goudali (G) and Goudali × Italian Simmental (SG) young bulls. Fifty G and SG reared entirely on native pasture in Northwest Cameroon and belonging to one farm were considered. Body weight (BW) of animals was recorded on-farm. After slaughter, the cold carcass weight and the fifth quarter composition were recorded, and the killing out percentage calculated on the reconstructed *ante mortem* BW (BWam). After 24 h a sample joint was removed from the 8th rib position, dissected in lean, fat and bone portion and the proximate composition was carried out. SG showed higher BW at farm, BWam, cold carcass weight and KO%, than G, but also a marked loss of weight during transfer and lairage time. SG had lower percentage of feet, tail, filled gut, and higher weight of rib steak, ribeye and percentage of lean tissue than G. Conversely, the LT composition was similar between breeds. Results suggest that performances and fat composition of LT of F1 crosses, SG, in comparison with the pure breed, G, could be further increased by improving the bull's transfer and pre-slaughter conditions.

Key Words – Crossbreeding, *Longissimus thoracis* m. composition, Tropical environment.

I. INTRODUCTION

Cattle, with a population of over six million, contribute 28% approx. of the total animal protein produced in Cameroon and there is a strong potential for live-animal export to neighbouring countries in the sub-region. However, the productivity of the principal breeds of cattle, under the prevailing production environment, have remained relatively low over the years because of absence of a well-defined and sustained plan for improving nutrition, genetic selection within and between breeding stock. The increasing demand for beef and milk are veritable opportunities for poverty alleviation and livelihood improvement. Against this background SODEPA in technical collaboration with the Breeders' Association of Italian Simmental (ANAPRI), is working on an on-farm trial to improve the animal production by crossbreeding Goudali with the Simmental. The Goudali, a *Bos indicus*, is a popular autochthonous beef-type with good growth rate and appreciable tolerance to endemic diseases such as trypanosomosis and tick-borne infections [1], while the Italian Simmental is a rustic, ecologically-friendly and dual-purpose *Bos taurus* breed. This controlled crossbreeding programme targets the establishment of a more productive stock with a relatively improved potential for beef and milk that can diversify and improve significantly the income of livestock farmers in Cameroon.

II. MATERIALS AND METHODS

Three hundred and thirty Zebu Goudali (G) cows were recruited to constitute a breeding herd based on the following criteria: be between 5 to 8 years old, with at least two successful parturitions, of good mothering instincts, clinically healthy, nursing a calf of 1-3 months at point of recruitment, and be in good body condition. The selected cows were individually identified by use of plastic ear-tags and corresponding rumen transponders and then cordoned off within 600ha of the 38000ha of Dumbo Ranch located at Latitude 060 42' N and Longitude 0100 25' E. They were organised into five artificial insemination breeding herds. Oestrus was synchronised and the cows bred using frozen 0.25cc straw-type doses of semen from 13 different Italian Simmental (IS) bulls. After calving each of the ISxG crossbred calf (SimGoud, SG) was identified by use of a plastic ear-tag and a corresponding rumen transponder.

To constitute a control against which the performances of the crossbred calves could be monitored, pure bred calves (G) born by Goudali cows on natural mount in the commercial ranch herds at about the same week with those produced by crossbreeding, were equally identified and subjected to the same nutritional plan (herbage grazed on Western Highland Plateau Savannah pasture+NaCl supplementation) and zoo-veterinary care, by introducing them immediately after calving in the artificial insemination breeding herds.

Figure 1. Representative sample of Goudali bulls at three years of age

Figure 2. Representative sample of SimGoud bulls at three years of age

Forty-two months after the first SG calving, 50 young male bulls of the two genotypes, from 20 to 41 months (Figs.1 and 2), were randomly selected in groups of 10 per experimental herd. The 25 SG young bulls were sired by five different IS bulls while the S ones originated from five different commercial herds of the ranch. The animals were weighed (body weight at farm, BWfarm) and and moved initially on-foot for 8 days over 208km to Bamenda and then loaded in unspecialised animal transport trucks, as is commonly practiced by cattle traders, to Douala in an 8hours drive over 306km. To alley stress the animals were rested for five weeks at the Douala Cattle market lairage during which they were grazed intermittently on native pastures on the outskirts of the town close to the market. After this the animals were slaughtered in the SODEPA industrial abattoir at Douala.

Immediately after slaughter, the fifth-quarter (FQ) components and the hot carcass weight (CW) were recorded and used to estimate the individual reconstructed *ante mortem* BW (BWam), the approximate empty body weight (EBW= BWam-filled gut) and the transfer losses (Tlosses,%= 100x[BWfarm- BWam]/BWfarm). After chilling for 24h, the half carcasses were weighted to obtain the cold carcass weight (CC) and the killing out percentage on the BWam (KO%). From the left side of carcass, a sample joint was removed from the 8th rib position, dissected in lean, fat, bone and other tissues portion and the proximate composition was carried out [2] on a sample of m. *Longissimus thoracis* (LT).

The normality of the data distribution was tested using the Kolmogorov-Smirnov test. The effect of genotype was evaluated by the analysis of covariance using 'genotype' (G *vs.* SG) as a fixed factor and 'age' as a covariate, an intra-class covariate when the intra-genotype coefficients were significantly different. In tables, the genotype means were adjusted to a covariate mean age of 31 months. The allometry coefficients were calculate by regressing the natural logarithm (ln) of the body component on the ln of the body (EBW or BWam).

III. RESULTS AND DISCUSSION

As expected, SG showed higher BWfarm than G ($P<0.05$; Table 1), with a four times higher growth rate between 20 to 41 months (9.46 *vs.* 2.57 kg/month). This result is due to the combination of additive and heterosis gene effects. In particular, considering that the F1 crosses are considered, the expected breed additive contributor and heterosis effect is 50% and 100% respectively. Demeke *et al*. [3], crossing Simmental breed with three different *Bos indicus* breeds in tropical Africa improved the yearling weight from 19 to 20%. However, many authors reported that heterosis effect is modulated by environment and production system [4,5].

Table 1 Weight of body, at farm and *ante mortem*, fifth quarter and cold carcass; losses during transfer and KO percentage of young bulls of different genotype

	Goudali (G)	SimGoud (SG)	MSE	Age coeff. G	Age coeff. SG
BWfarm (kg)	220[b]	354[a]	3.22	2.57[**]	9.46[**]
Tlosses (%)	-4.4[b]	6.1[a]	.763	0.48[**]	0.81[**]
BWam (kg)	228[b]	330[a]	2.39	2.26[**]	5.52[**]
FQ (kg)	104[b]	139[a]	1.27	0.56	0.80[*]
CC (kg)	103[b]	159[a]	1.61	0.89[*]	4.82[**]
KO (%)	45.1[b]	48.1[a]	.347	0.10	0.54[**]

[a,b] or [*] P<0.05; [**]: P<0.01

It is interesting to note that SG, despite having higher BWam than G (P<0.05), showed a marked loss of weight during transfer and lairage time (-6%). Conversely, G, during this period was able to increase BW of 4.4%. These results could be due to the higher nutrient requirements and/or to the lower adaptability to transfer condition of the crosses, SG, in comparison with the pure breed, G. At slaughter, SG showed higher carcass weight and KO% than G (P<0.05; Table 1) confirming the superiority of the F1 crosses in comparison with the pure breed. In a survey performed in Cameroon that involved G bulls from different production systems, Nfor *et al.* [6] recorded a hot carcass weight of 152 kg at 4 years. Williams *et al.* [7] highlighted a positive heterosis effect on carcass weight crossing *Bos taurus* and *Bos indicus* breeds.

Table 2 Fifth quarter composition (%BWam) and allometry coefficients in relationship with EBW

	Goudali (G)	SimGoud (SG)	MSE	Allometry G	Allometry SG
Head	5.75	5.53	.067	0.85[**]	0.85[**]
Feet	3.24[a]	2.93[b]	.069	0.29[*]	0.31[*]
Tail	1.18[A]	0.84[B]	.042	0.60	0.57
Skin	3.99	3.76	.138	1.33[**]	1.28[**]
Pluck	3.11	3.36	.082	0.78[**]	0.80[**]
Filled gut[1]	28.03[A]	25.02[B]	.527	0.18	0.22

[1]Allometry coefficient on BWam;
[A,B] or [**]: P<0.01; [a,b] or [*]: P<0.05

Considering the fifth quarter composition, SG showed a significantly lower percentage of feet, tail and filled gut, but similar percentage of head, skin and pluck (P>0.05) than G as reported in Table 2. As expected, the allometry coefficient was lower than 1 for head, feet and pluck in both experimental groups indicating that this parts are early maturing respect to EBW (Table 2). Also filled gut showed a diminutive allometry in relationship with BWam.

Table 3 Weight and tissue composition of the sampling cut (8th-9th rib section) from young bulls of different genotype

	Goudali	SimGoud	MSE
Rib steak weight (g)	510[b]	760[a]	17.2
Ribeye muscle (g)	102[b]	173[a]	4.96
Lean (%)	66.3[b]	68.9[a]	.358
Fat (%)	3.9	2.8	.363
Bone (%)	24.6	24.3	.765

[a,b]: P<0.05

As reported in Table 3, SG showed higher weight of rib steak and ribeye muscle at 8th-9th rib section level than G (P<0.05). The covariate, age, was significantly related to weight of rib steak and ribeye muscle for SG (21.5 and 5.9 g/month respectively, P<0.01), but not for G (1.7 and 0.7 respectively, P>0.05; data not reported in Tables). These results indicate that SG had a greater growth than G. In particular, considering the sampling joint composition, SG had higher percentage of lean tissue and similar percentage of fat and bone tissue than G (P<0.05; Table 3). Corazzin *et al.* [8] in a study that considered Simmental young bulls fed with concentrate, reported a sampling joint composition of 64.7% meat, 14.0% fat and 17.0% bone. Perotto *et al.* [9] crossing Nellore, a *Bos indicus* breed, with Simmental observed an increase, despite not significant, of 2.7% of the percentage of lean meat of sample joint at 12th rib. Theuissen *et al*. [10], crossing *Bos taurus* and *Bos indicus* breeds observed an heterosis effect of +0.8% on meat yield that was estimated considering the dissection of sample joint at 8th-10th rib level. Considering the sample joint and KO results, it could be speculated that SG had better carcass conformation at slaughter than G.

Table 4 *Longissimus thoracis* composition (g/100g fresh meat) of young bulls of different genotype

	Goudali	SimGoud	MSE
Moisture	76.6	76.0	.227
Protein	20.1	20.5	.248
Fat	.60	.76	.053
Ash	1.06	1.06	.017

LT composition is shown in Table 4. Differences between G and SG were not found (P>0.05). Marshall [11], reviewing the effects of different breed crosses, reported an average positive heterosis effect of 3.8% for marbling. Conversely, Gama et al. [4], crossing *Bos taurus* and *Bos indicus* breeds in pasture finishing conditions, showed a

significant heterosis effect for moisture, +1.4%, but not for fat, protein and ash. The above cited Authors explained that heterosis effect is strongly influenced by the animals' diet. Consequently, in our study, the lack of additive and heterosis effects at slaughter on meat fat content could be due to the severe transfer conditions of bulls from farm to slaughterhouse that have caused a probable reduction of the final fat level in muscle. Indeed, the average fat level was low, 0.68%, much lower than the value reported by Nfor *et al.* [12], 1.34% in G reared in Cameroon and with similar feeding conditions, but transported to slaughterhouse by truck. The average protein level found, 20.3%, fell within the range proposed for beef by Muchenje *et al.* [13], 20.0-22.9%, but it was lower than those showed by Salifu *et al.* [14] in zebu Fulani, 21.7%, and by Nfor et al. [6] in G bulls, 22.1%, both reared on natural pasture in tropical environment.

IV. CONCLUSION

G pure breed zebu showed much lower *in vivo* and at slaughter performance than their crosses with Simmental breed, probably because of additive and heterosis effects that interact with the severe rearing conditions of the animals. The results of this study indicate that, in order to maximize the crossbreeding effect, the bulls' transfer and pre-slaughter conditions should be improved.

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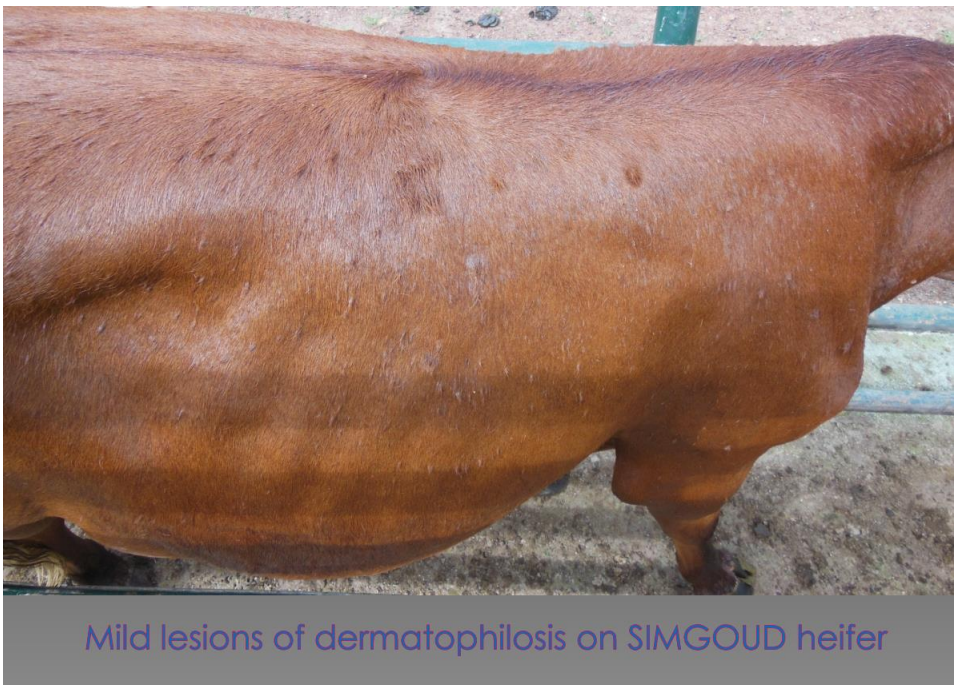
7.4 Annex 4- Assessment of clinical dermatophilosis, photo report



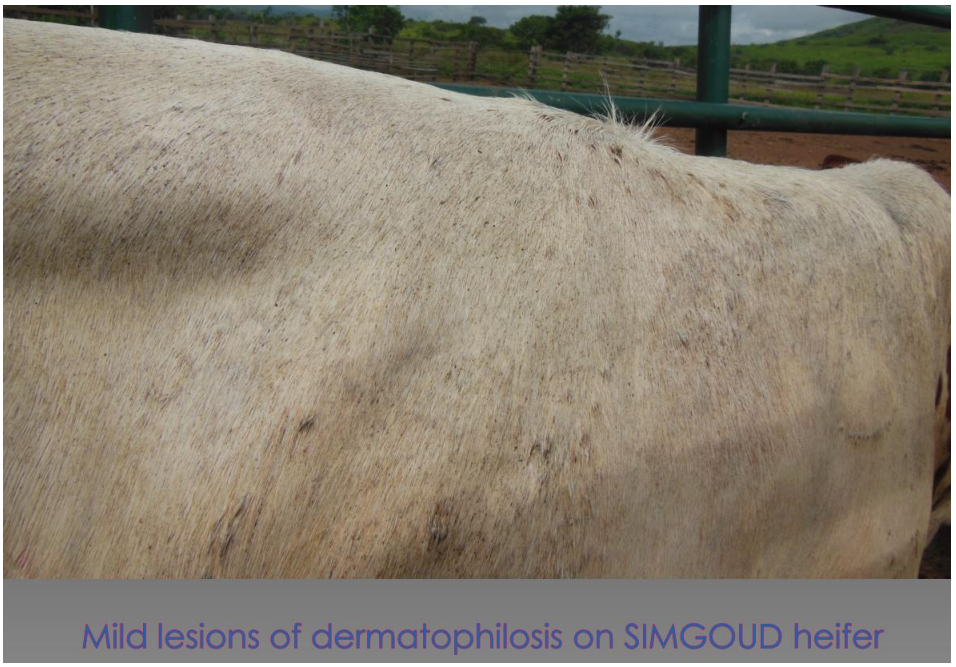


Figure 1.a and 1.b. MILD, lesions were confined to just a few raised hair with underlying papules confined to the neck, face and dewlap regions without matting.

Figure 2. MODERATE, the lesions, in addition to the situation of mild, extended to the back with matting of hair on affected areas.

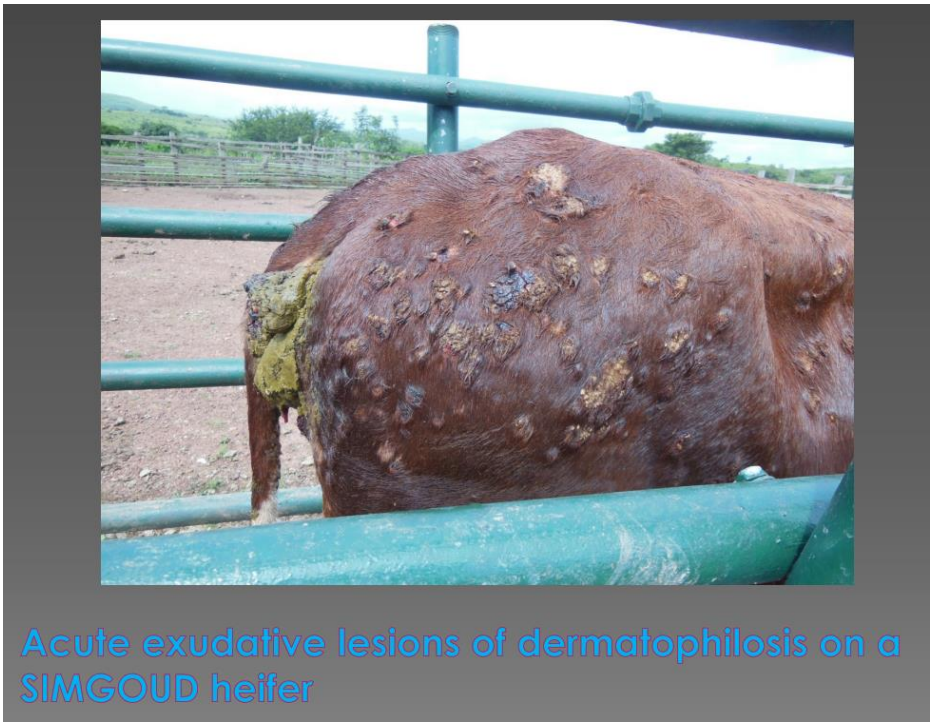


Figure 3. SEVERE, the lesions were generalized matting and encrusted involving wide areas of the skin, extremities, udder, and testicles and were life-threatening with a need for culling.

Figure 4a and 4b. Animals with-out clinical lesions related to dermatophilosis were considered UNAFFECTED.

7.5 Annex 5- Jatropha curcas cultivation in Dumbo ranch and diffusion in Misaje Communality.

Nursery

Plant distribution

Plant cultivation

Seed destination

Seeds of Jatropha plant found at a village about 30km from the Dumbo ranch growing wild.

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9. Main activities

Courses

- Training on Artificial Insemination
- Use of ultrasound in the management of reproduction cows
- Comprehensive course on bovine assisted Reproductive biotechnology (2012). The International Embryo Transfer School, Senatobia, Memphis; USA
- Meat Science laboratory Procedures: Beef and Pork Carcass Evaluation

Participation in Congresses

29th European Simmental Federation Congress, Udine, Italy May, 2011

63rd Annual Meeting of the European Federation of Animal Science, Bratislava. August 2012

International Congress of Meat Science and Technology, Clermont Ferrand; August 2015

Presentation at congresses

Results of a Crossbreeding Program of Autochthonous Goudali (Zebu) Cattle of Cameroon with Italian Simmental. Book of abstracts 29th European Simmental Federation Congress. ANAPRI, Udine, Italy. 183-202.

Poster presentation: W.O. Bessong, M. Corazzin, E. Saccà, C. Biondokin, G. Menta, E. Piasentier, "Carcass and meat characteristics of young bulls from local Goudali (Zebu) cattle of Cameroon and its crosses with Italian Simmental". 61st International Congress of Meat Science and Technology (ICOMST), August 23-28, 2015, Clermont-Ferrand, France.

Workshops

Embryo Transfer under tropical conditions; Medea, Algeria. Arab Fund for Technical Assistance

Consultative workshop on the design of crossbreeding and selection programme for cattle in Cameroon

Coordination of trainings on Bovine Artificial Insemination UNIUD-SODEPA, Jakiri NWR Cameroon

Publications

- *Esemu SN*, *Besong WO*, *Ndip RN*, *Ndip LM* (2012). Prevalence of Ehrlichia ruminantium in adult Amblyomma variegatum collected from cattle in Cameroon.. *Exp Appl Acarol. 26th July 2012*

-*Ngoula F, Tarla D. N, Kenfack A, Bayemi P. H, Bessong* W.O (2014). Effect of Egg Yolk Concentration in Semen Extender, pH Adjustment of Extender and Semen Cooling Methods on Bovine Semen Characteristics., Tadondjou Cyrille D'Alex, Kamtchouing Pierre and Tchoumboué Joseph. Global Veterinaria 12 (3): 292-298, 2014

- *Fonteh F A, Bawe N M, Tuncha N P, Bessong O W, Muluh M E and Piasentier E* 2015**:** Influence of production system on the quality of Gudali beef in Cameroon. *Livestock Research for Rural Development. Volume 27, Article #29.* Retrieved February 3, 2016, from http://www.lrrd.org/lrrd27/2/font27029.htm