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# VALORISATION DES SOUS-PRODUITS DE LA GRAINE DE *JATROPHA CURCAS* L. EN PRODUCTION DE POULETS AU SÉNÉGAL

# VALORIZATION OF *JATROPHA CURCAS* L. SEED BY-PRODUCTS IN POULTRY PRODUCTION IN SENEGAL

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**THÈSE PRÉSENTÉE EN VUE DE L'OBTENTION DU GRADE DE DOCTEUR EN SCIENCES VÉTÉRINAIRES – ORIENTATION SANTÉ ET PRODUCTIONS ANIMALES**

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**A DIEU LE PÈRE TOUT-PUISSANT**

« *Ma force et mon chant, c'est le Seigneur ; Il est pour moi le salut* » Ps. 117, 14

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**RÉSUMÉ / SUMMARY**

**RÉSUMÉ**

Cette étude a évalué les effets du tourteau de l'amande de *Jatropha curcas* incorporé en alimentation des volailles au Sénégal. Tous les essais ont été conduits au niveau de la station expérimentale de l'Ecole Nationale Supérieure d'Agriculture (ENSA) de l'Université de Thiès (Sénégal). Il en est de même pour toutes les analyses chimiques qui ont été réalisées au laboratoire de bromatologie du Département des Productions Animales (DPA) de l'ENSA.

La première partie de ce travail a fait une description des composés toxiques et antinutritionnels présents dans les tourteaux de la graine de *J. curcas* dont les plus importants sont les esters de phorbol caractéristiques des *Euphorbiaceae*, connus pour leur action inflammatoire et la curcine, une toxalbumine qui se manifeste par une action irritante. D'autres composés sont également présents et interfèrent plus ou moins avec les processus digestifs chez les animaux. Différents traitements ont été appliqués, selon différents niveaux de réussite, pour réduire ou éliminer ces composés toxiques ou antinutritionnels permettant ainsi la valorisation de ces tourteaux en alimentation des animaux. Un accent particulier a été mis sur les procédés biologiques de détoxification pour, non seulement une réduction significative de ces composés toxiques, mais aussi une amélioration de la qualité nutritionnelle du tourteau.

La seconde partie de ce travail a porté sur l'étude de l'effet du tourteau de l'amande de *J. curcas* obtenu par déshuilage chimique, incorporé à du maïs selon des proportions de 4, 8 et 12% et distribué à des poulets de chair en fin de phase de croissance afin d'évaluer leur ingestion, leur gain pondéral et la digestibilité de la ration. Ainsi, pour les rations distribuées, l'ingestion alimentaire a été influencée par l'incorporation du tourteau de l'amande de jatropha conduisant à une baisse de l'ingestion et du gain de poids chez les animaux qui ont reçu les quantités les plus importantes de jatropha malgré l'absence de signes de toxicité et de mortalité. La digestibilité des principaux nutriments a aussi été affectée par l'incorporation du tourteau de l'amande de jatropha ainsi que la digestibilité différentielle de ceux-ci. La faible digestibilité, notamment protéique s'est expliquée par la faible disponibilité des protéines du tourteau incorporé au maïs mais aussi à la présence des facteurs antinutritionnels et toxiques encore présents dans les rations utilisées.

La troisième partie du travail a présenté les résultats obtenus de deux expérimentations qui ont été menés successivement. La première a étudié l'effet du tourteau de l'amande de jatropha obtenu par un déshuilage chimique et incorporé dans une ration selon des proportions de 4 et 8%, sur les performances de volailles de type chair en phase de croissance/finition. La seconde expérimentation a étudié les effets du même tourteau qui a subi un traitement thermique puis incorporé à 8%, sur les performances de poussins de chair d'un jour. Dans l'un comme l'autre essai, les ingestions et les gains de poids ont été affectés par l'incorporation du tourteau de l'amande de jatropha. Il en est de même pour les taux de mortalités enregistrées malgré l'absence de signes visibles de toxicité. Ces expérimentations nous ont permis de confirmer d'autres observations qui ont montré qu'un simple déshuilage de l'amande de graines de jatropha et qu'un traitement thermique appliqué ensuite sur celle-ci, même si elle entraîne une réduction significative de certains composés antinutritionnels, ne permet vraisemblablement pas de supprimer tous les facteurs toxiques qui peuvent encore interférer avec la prise alimentaire et le gain de poids des animaux.

Ces observations n'ont pas été confirmées dans la quatrième partie qui a étudié les effets du tourteau de l'amande de graines de jatropha obtenu par déshuilage chimique, objet d'un traitement thermique puis biologique avec *Aspergillus niger* et incorporé à 8% dans une ration, sur les performances de poussins de chair d'un jour. En effet, les ingestions, les gains de poids ainsi que la viabilité des animaux n'ont pas été affectés par l'incorporation du jatropha.

Ces études successives ont été parmi les premières qui ont intéressé une même espèce animale à différents niveaux de développement en se basant sur un produit dont la qualité nutritionnelle est avérée mais qui présente des facteurs toxiques et antinutritionnels n'ayant pu être complètement éliminés en dépit des différents traitements appliqués. L'application des traitements thermiques a sans doute permis de réduire certains composés antinutritionnels (curcine et inhibiteur de trypsine) mais sans que cela n'améliore l'ingestion et les gains de poids. En considérant la réaction des animaux soumis aux rations alimentaires, les traitements biologiques combinés aux traitements thermiques ont sans doute permis de baisser la toxicité du tourteau de jatropha ce qui a agi sur la prise alimentaire des animaux améliorant ainsi l'ingestion des aliments et donc les gains de poids.

Cette étude a permis de montrer que, malgré son niveau nutritionnel, le tourteau de l'amande de *J. curcas* a présenté des limites dans son utilisation comme matière première pouvant intégrer une ration destinée à des poulets de chair au Sénégal mais des espoirs restent permis avec les traitements biologiques.

## SUMMARY

This study evaluated the effects of *Jatropha curcas* kernel meal incorporated in poultry feed in Senegal. All experimentations were conducted in the experimental station of the National Superior School of Agriculture (*ENSA*) of Thies University (Senegal). In the same way, all chemical analyses were performed in the feed science laboratory of the Department of Animal Production of *ENSA*.

The first part of this study was a description of toxic and anti-nutritional compounds present in *J. curcas* seed meal, which most important are phorbol esters, characteristics of *Euphorbiaceae*, known for its inflammatory effects, and curcin, a toxalbumin which manifested by an irritant action. Other compounds, also present, interfere more or less with digestive process in animals. Different treatments were applied, with different levels of success, to reduce or eliminate these toxic and anti-nutritional compounds for the valuation of this meal in animal feed. A particular emphasis was placed on the biological treatment, not only for a significant reduction of these compounds but also to improve the nutritional quality of the meal.

The second part of this work has focused on the study of the effect of the *J. curcas* kernel meal obtained by chemical de-oiling, incorporated in corn at 4, 8, and 12% and distributed to broiler chickens at the end their growth phase to assess their feed intake, their daily weight gain and diet digestibility. For all diets, feed intake was inversely influenced by the incorporation on the jatropha kernel meal, leading a weight gain decrease in animals that received the largest amounts of jatropha despite the absence of toxicity signs and mortality. Similarly, the apparent digestibility of each nutrient has been affected by the incorporation of jatropha kernel meal as well as the differential digestibility thereof. The low digestibility, especially protein was explained by the low availability of meal proteins incorporated corn, but also the presence of anti-nutritional and toxic factors still present in the diets used.

The third part of this work has presented results of two experimentations conducted successively. The first one studied the effect of jatropha kernel meal obtained by a chemical de-oiling, incorporated in diets at 4 and 8%, on broiler chickens performance during growing/finishing. The second experimentation studied the effect of the same cake that was heat treated before incorporated in diets

at 8%, on day-old broiler chicks' performance. In one as the other experimentation, feed intake and daily weight gain were affected by the incorporation of jatropha kernel meal. It was the same for the total mortality rates recorded despite the absence of visible signs of toxicity. These experimentations confirmed other experimentations have shown that the de-oiling of jatropha kernel meal followed by heat treatment, even if it caused a significant reduction of anti-nutritional compounds, did not remove all the toxic factors that may even interfere with feed intake and weight gain of animals.

These observations were not confirmed in the fourth part, which studied the effects of jatropha kernel meal obtained by chemical de-oiling, subject to heat treatment then biological treatment with *Aspergillus niger* and incorporated in diets at 8%, on day-old broiler chicks performance. Indeed, the feed intake, the weight gain as well as the viability of animals were not affected by the incorporation of jatropha.

These successive studies were among the first which interested the same animal species at different levels of development based on a product whose nutritional quality is proven, but has toxic and anti-nutritional factors could not be completely eliminated despite different treatments applied. The application of heat treatment may have helped to reduce some anti-nutritional compounds (curcin and trypsin inhibitor) but without improve feed intake and weight gain. Considering the reaction of animals subjected to diet, biological and heat treatments may have reduced the toxicity of jatropha kernel meal, which has afected the feed intake of animals and thus the weight gain.

This study showed that, despite its nutritional level, *J. curcas* kernel meal presented limitations in its use as a raw material that can integrate a ration for broilers in Senegal but hopes remain with biological treatments.

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## AVANT-PROPOS

Cette thèse est structurée sous la forme d'une compilation d'articles scientifiques qui ont été publiés ou acceptés par les revues scientifiques suivantes : *Biotechnologie Agronomie Société et Environnement (BASE)*, *Revue Africaine de Santé et des Productions Animales (RASPA)*, *Tropical Animal Health and Production*, *Tropicultura.*

Cette étude sur les possibilités d'incorporation du tourteau de *Jatropha curcas* en alimentation animale comporte 5 chapitres après une introduction générale qui détermine le contexte et les objectifs de l'étude.

Le premier chapitre présente une revue bibliographique sur les principaux composés toxiques et antinutritionnels présents dans la graine de *Jatropha curcas* (article 1) et sur la détoxification biologique du tourteau de *J. curcas* (article 2). Ces revues ont fait la compilation des informations sur la nature des composés toxiques et antinutritionnels, leur mode d'action, leurs effets dans les organismes animaux ainsi que sur les procédés utilisés pour les éliminer notamment biologiques pour une utilisation en alimentation animale.

Le second chapitre est accès sur l'étude de la digestibilité de la digestibilité apparente et différentielle du tourteau de l'amande de *J. curcas* obtenu par un déshuilage chimique, ajouté à du maïs puis distribué à des poulets de chair en fin de phase de croissance (article 3).

Le troisième chapitre étudie l'effet du tourteau de l'amande de *J. curcas* obtenu par un déshuilage chimique, incorporé d'une part dans une ration destinée à des poulets de chair en phase de croissance et d'autre part, subissant un traitement thermique avant d'être incorporé dans une ration destinée à des poussins d'un jour (article 4).

Le quatrième chapitre est relatif à l'étude de l'effet du tourteau de l'amande de *J. curcas* obtenu par un déshuilage chimique, subissant un traitement thermique puis biologique avec *Aspergillus niger* puis incorporé dans une ration destinée à des poussins d'un jour (article en rédaction).

Une analyse synthétique et intégrée de l'ensemble des résultats obtenus a été présentée au niveau de la discussion générale et des perspectives au cinquième chapitre. Elle met évidence les limites de l'utilisation du tourteau de *J. curcas* en alimentation des poulets de chair malgré sa qualité nutritionnelle.

# INTRODUCTION GÉNÉRALE

*Jatropha curcas* L. est une *Euphorbiaceae,* appelée pourghère, grand pignon d'Inde, médicinier purgatif en français ; *jatropha*, *physic nut* en anglais ; *prugueria*, *ricino major* en portugais, *piñón de tempate*, *jatrofa* en espagnol (Schmelzer & Gurib-Fakim, 2008) et *tabanani* au Sénégal. Elle doit son étymologie aux racines grecques *iatrós* (médecin) et *trophê* (nourriture) en raison des nombreuses utilisations thérapeutiques traditionnelles dont elle fait l'objet (Parawira, 2010).

Originaire d'Amérique centrale, du Texas au Mexique, *J. curcas* a été répandue dans toutes les régions tropicales et subtropicales et sa diversité géographique ne semble pas représenter une diversité génétique car les accessions du Sénégal par exemple, sont étroitement liées à celles de l'Inde (Ndir et al., 2013 ; Ouattara et al., 2014). La plante s'est naturellement adaptée à des climats chauds et humides ainsi qu'à des sites pauvres et secs (Montoya & Tejeda, 1989). Sa disposition à croître dans les zones sèches, avec des mécanismes d'adaptation à la sécheresse ainsi que sur des sols dégradés peu aptes à l'agriculture, la rend particulièrement attrayante (Brittaine & Lutaladio, 2010). Elle se développe mieux sur des sols bien drainés et aérés mais s'adapte bien à des conditions marginales c'est-à-dire des sols à faible teneur en éléments nutritifs (Jongschaap et al., 2007). Au Sénégal, la plante qui présente une grande variabilité phénotypique se développe toutefois mieux dans les zones humides où elle est largement distribuée (Ouattara et al., 2013).

L'espèce se présente sous forme d'un arbuste caducifolié en cas de stress hydrique, à tiges légèrement succulentes, pouvant atteindre des hauteurs comprises entre 5 et 8 m. Ses branches sont épaisses, cassantes et dressées à cime ouverte avec des feuilles alternes, simples, généralement palmatilobées (Dehgan & Webster, 1979). Comme toutes les *Euphrobiaceae*, elle présente un latex aqueux à blanchâtre. La propagation se fait par graines, avec une germination qui dure une dizaine de jours (Heller, 1996), variable en fonction des graines (Samba et al., 2007) et dont la transplantation de plantes sauvages présente l'inconvénient de générer des plantes hétérogènes (Medza Mve et al., 2010). Lorsque les conditions sont favorables (Terren et al., 2012), elle se multiplie également par bouturage réalisé avec des rameaux qui se dessèchent peu en raison de la couche de cire qui les protège (Henning, 2003) mais qui présentent plus tard un système radiculaire superficiel pouvant rendre la plante sensible à la sècheresse.

La croissance végétative se produit durant la saison humide avec une floraison déclenchée par les pluies ; les graines sont produites dans la première ou la deuxième année (Henning, 2008). Pendant la saison sèche, la plante perd la plupart de ses feuilles réduisant ainsi la perte d'eau par transpiration (Kheira & Atta, 2008). Dans de telles conditions, elle peut vivre entre 30 et 50 ans. Après la pollinisation, les inflorescences forment des grappes de fruits ovoïdes qui renferment deux à trois graines en moyenne par fruit (Kochhar et al., 2008). Ces graines, qui représentent 60 à 65% du poids du fruit sec (**Figure 1**), sont ovales, de couleur brun foncé à noir et enveloppées d'une coque sous laquelle se trouve un tégument intérieur recouvrant l'amande (environ 60% de la graine) (Vyas & Singh, 2007).

Par la puissance de son système racinaire, *J. curcas* est utilisée pour réduire l'érosion éolienne (Achten et al., 2007) ainsi que pour stabiliser des dunes et des pentes (Francis et al., 2005). La plante est aussi utilisée comme haie vive répulsive pour la protection des habitations et des terres agricoles contre les dommages causés par le bétail (Kumar & Sharma, 2008). On lui attribue, par ailleurs, des propriétés topiques, anti-infectieuses, coagulantes et toutes les parties de la plante peuvent être employées telles quelles ou en décoction, en médecine traditionnelle ou à des fins vétérinaires (Berhaut, 1975 ; Heller, 1996 ; Osoniyi & Onajobi, 2003 ; Beyra et al., 2004).

La graine de *J. curcas* contient une huile visqueuse dont la fraction totale se trouve dans l'amande et qui est utilisée comme matière première pour la fabrication de savons et d'huile de combustion (Schmelzer & Gurib Fakim, 2008). Cette huile, riche en acides oléique et linoléique (72 – 84% des acides gras) (Banerji et al., 1985) présente les qualités optimales pour produire un carburant diesel (Kureel, 2006 ; Vaitilingom, 2007 ; Akbar et al., 2009) par divers procédés (Berchmans & Hirata, 2008 ; Parawira, 2010). C'est ainsi que Francis et al. (2005) avaient estimé que *J. curcas*, par sa production d'huile et ses potentialités d'adaptation à des zones arides, pouvait constituer une source d'énergie renouvelable exceptionnelle tout en améliorant la qualité de l'environnement. Le péricarpe du fruit est toxique (Ferreira et al., 2012) mais la graine est cependant considérée comme la partie la plus toxique de la plante pour diverses espèces animales (Adam & Magzoub, 1975 ; Abdu-Aguye et al., 1986 ; Blumberg, 1988 ; Liberalino et al., 1988 ; Mampane et al., 2006), notamment chez les volailles (El Badwi et al., 1995 ; Sirisha et al., 2008 ; Sirisha et al., 2009 ; Ojo et al., 2013) provoquant

de l'inappétence, des douleurs abdominales, des vomissements, de la diarrhée profuse verdâtre, des difficultés respiratoires, une restriction des mouvements et un impact négatif sur certains paramètres hématologiques. Les variétés trouvées en Afrique et en Asie produisent des graines qui sont toxiques pour les humains et les animaux alors que certaines variétés trouvées au Mexique et en Amérique centrale sont connues pour être non toxiques (Makkar et al., 1998a ; Makkar et al., 1998b ; Baldini et al., 2014) et pourraient être exploitées (King et al., 2009).

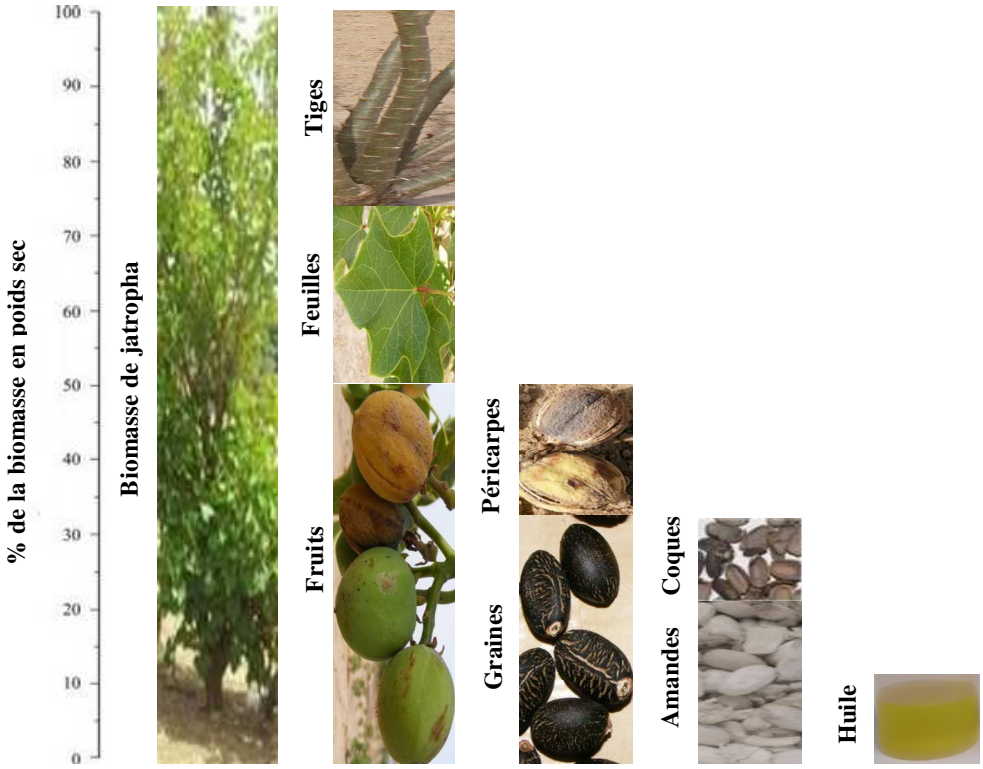


**Figure 1 :** Répartition de la biomasse de jatropha (% de la matière sèche).

Inspirée de Navarro-Pineda et al. (2016)

A l'issue de la trituration de la graine selon divers procédés (Pradhan et al., 2009 ; Beerens, 2007), un tourteau, qui représente parfois plus de 50% de la graine (Becker, 2009), est obtenu comme résidu. Ce dernier présente des composés toxiques et antinutritionnels (Makkar et al., 1997 ; Abou-Arab & Abu-Salem, 2010 ; Saetae & Suntornsuk, 2010). Il constitue un excellent engrais organique avec une teneur en azote similaire ou meilleure que le fumier de poulet (Patolia et al., 2007 ; Peace & Aladesanmi, 2008 ; Ali et al., 2010). Compte tenu de son potentiel nutritionnel, notamment sa teneur en protéines (Montoya & Tejeda, 1989 ; Aderibigbe et al., 1997 ; Achten et al., 2008 ; Devappa & Swamylingappa,

2008 ; Lago, 2009), il pourrait également être incorporé en alimentation animale (Makkar & Becker, 1999 ; Belewu et al., 2010 ; Sumiati et al., 2011).

D'ailleurs le profil en acides aminés (**Tableau 1**) a montré une grande similitude entre les tourteaux provenant d'une variété dite « toxique » et celle dite « non toxique » avec, pour la plupart des acides aminés, des niveaux sensiblement supérieurs aux normes protéiques de référence recommandées par la FAO pour un enfant de 2 à 5 ans (Makkar et al., 1998a).

| **Acides aminés** | **Composition en acides aminés (g 16g$^{-1}$ d'azote)** | | |
|---|---|---|---|
| | **Variété cap-verdienne** | **Variété mexicaine** | **Référence de la FAO** |
| Lysine | 4,3 | 3,4 | 5,8 |
| Leucine | 6,9 | 7,5 | 6,6 |
| Isoleucine | 4,5 | 4,9 | 2,8 |
| Méthionine | 1,9 | 1,8 | 2,5 |
| Cystine | 2,2 | 1,6 | |
| Phénylalanine | 4,3 | 4,9 | 6,3 |
| Tyrosine | 3,0 | 3,8 | |
| Valine | 5,2 | 5,3 | 3,5 |
| Histidine | 3,3 | 3,1 | 1,9 |
| Thréonine | 4,0 | 3,6 | 3,4 |
| Sérine | 4,8 | 4,8 | - |
| Acide glutamique | 14,7 | 15,9 | - |
| Acide aspartique | 9,5 | 9,9 | - |
| Proline | 5,0 | 3,8 | - |
| Glycine | 4,9 | 4,6 | - |
| Alanine | 5,2 | 4,9 | - |
| Arginine | 11,8 | 12,9 | - |
| Tryptophane | 1,3 | - | 1,1 |

**Tableau 1 :** Composition comparative en acides aminés entre le tourteau de graine de *Jatropha curcas* d'une variété cap-verdienne (toxique), d'une variété mexicaine (non toxique) et les normes recommandées par la FAO selon Makkar et al. (1998a).

Au Sénégal, la filière avicole a connu ces dernières années un développement important de son secteur semi-industriel et industriel mais aussi de celui familial, très répandu en milieu rural (Traoré, 2014). Les effectifs ont été d'environ 44,2 millions de têtes en 2012 (ANSD, 2015), permettant de fournir environ 36% de la production nationale de viande et d'abats. L'alimentation est déterminante dans le développement de ce secteur, aussi bien moderne que traditionnel. Elle représente environ 75% des coûts de production (Bebay, 2006), mais reste fortement tributaire de matières premières dont la plupart sont importées (Cothenet & Bastianelli, 1999). Pour impulser le développement de ce secteur, le gouvernement de la république du Sénégal avait, en 2013, proposé entre autres mesures, de produire

localement du maïs pour l'aviculture et faciliter la contractualisation des relations commerciales entre producteurs de maïs et provendiers, mais aussi d'accorder la priorité de vente des tourteaux d'arachide produits localement aux provendiers (MEPA, 2014). Plus largement, la recherche s'est aussi développée pour la valorisation de produits et sous-produits locaux, performants dans les conditions climatiques locales et pouvant être intégrés à des formules alimentaires (Llopis et al., 1981 ; Dahouda et al., 2009 ; Tendonkeng et al., 2009 ; Ayssiwede et al., 2010 ; Diaw et al., 2012).

Le Sénégal, à l'instar de plusieurs pays en développement, qui a été confronté à l'augmentation de la demande en énergie pour les zones rurales, devait également faire face à une pression économique et environnementale sur des terres agricoles particulièrement (Brittaine & Lutaladio, 2010). C'est ainsi que *Jatropha curcas* L., plante oléagineuse sous-utilisée, cultivée sur des terres marginales dans des conditions de sécheresse, a présenté non seulement une opportunité de culture énergétique, mais aussi un moyen de réduction de la pauvreté des populations rurales (MDRA, 2007). En effet, la graine permet de produire un biodièsel non polluant destiné à l'éclairage par lampe tempête ou à la fabrication de savon, et un tourteau, comme sous-produit, pouvant servir de fertilisant (Barbier et al., 2012). De nombreux projets se sont développés et ont principalement été axés sur l'amélioration génétique de la plante ainsi que sur son introduction en association avec d'autres spéculations vivrières.

C'est dans ce cadre que la présente thèse a été initiée pour déterminer, au Sénégal, les possibilités d'utilisation de l'amande de *J. curcas* incorporé dans l'alimentation de monogastriques, notamment de volailles, après avoir subi des traitements en vue d'une détoxification.

# CHAPITRE I

## REVUE BIBLIOGRAPHIQUE

Cette revue bibliographique constitue l'aggrégation de deux articles de synthèse bibliographique qui ont pratiquement traité du même sujet.

1. **Principes toxiques, toxicité et technologie de détoxification physico-chimique ainsi que biologique de la graine de *Jatropha curcas* L.**

**Article 1 – Principes toxiques, toxicité et technologie de détoxification de la graine de *Jatropha curcas* L. (synthèse bibliographique).**

Publié dans la revue BASE (Biotechnologie, Agronomie, Société et Environnement)

Volume 16 (2012), numéro 4, pp 531-540

Thierry Daniel Tamsir NESSEIM, Marianne FILLET, Guy MERGEAI, Abdoulaye DIENG & Jean-Luc HORNICK

**Article 2 – Toxicité et détoxification biologique du tourteau de *Jatropha curcas* L. pour une utilisation en alimentation animale : Synthèse bibliographique.**

Publié dans la revue RASPA (Revue Africaine de Santé et des Productions Animales)

Volume 12 (2014), numéro 3-4, pp 143-149

Thierry Daniel Tamsir NESSEIM, Abdoulaye DIENG, Guy MERGEAI, & Jean-Luc HORNICK

### 1.1. Introduction

*Jatropha curcas* L. (*Euphorbiaceae*) est un arbuste originaire d'Amérique, largement distribué dans les zones tropicales et subtropicales d'Afrique et d'Asie. Ses graines sont riches en composés lipidiques dont la teneur moyenne représente 36,3% de la matière sèche (MS) de la graine entière (Ginwal et al., 2004) ou 56,75% de la MS de l'amande (Makkar et al., 1998a). L'huile, considérée comme un combustible potentiel de substitution au diesel, pourrait être utilisée dans les moteurs à explosion soit directement, soit après raffinage. Le tourteau issu de la trituration des graines est utilisé comme fertilisant organique (Staubmann et al., 1997) ; il pourrait être valorisé en alimentation du bétail car il présente un niveau en protéines brutes pouvant atteindre 61,2% de la MS, valeur supérieure à celle du tourteau de soja (45,7% de la MS) (Makkar et al., 1998a). Mais cette utilisation est limitée par la présence de certains métabolites toxiques et facteurs antinutritionnels.

L'objectif de cette synthèse est de passer en revue les métabolites toxiques et antinutritionnels majeurs accumulés dans la graine de jatropha, d'en évaluer leur toxicité et de recenser les techniques utilisables pour réduire leur présence dans les tourteaux.

### 1.2. Inventaire des principales toxines

L'analyse chimique des graines de *J. curcas* a mis en évidence la présence de substances toxiques pour les animaux ou l'homme, mais aussi de facteurs antinutritionnels (**Tableau 1**). Parmi ces composés, on trouve des esters de phorbol (Makkar et al, 1997 ; Makkar et al., 1998a ; Martínez-Herrera et al., 2006), des molécules – notamment la curcine – à activité lectinique, un inhibiteur de la trypsine et des saponines (Aderibigbe et al., 1997). Certains composés sont des métabolites secondaires qui, contrairement aux métabolites primaires, ne participent pas directement au développement des plantes, mais peuvent intervenir dans leur protection contre les agressions des pathogènes et des herbivores, ou dans leur reproduction par attraction de pollinisateurs (Raven et al., 2003). On identifie trois types principaux de métabolites secondaires : les terpénoïdes, les alcaloïdes et les molécules phénoliques. Les esters de phorbol font partie des terpénoïdes et sont les composés les plus toxiques dans *J. curcas* et les saponines font partie des composés phénoliques. Par contre, curcine et inhibiteur de trypsine sont des protéines (métabolites primaires).

| **Matériel végétal** | **Composés toxiques et antinutritionnels** | | | | |
|---|---|---|---|---|---|
| | **Amande** | | **Tourteau** | | |
| | **Esters de phorbol (%MS)** | **Inhibiteur de trypsine (%MS)** | **Saponines (%MS)** | **Phytates (%MS)** | **Curcine/activité lectine (mg MS.ml$^{-1}$)** |
| ***Jatropha* « toxique »** | 0,2 | 2,1 | 2,3 | 9,4 | 102,0 |
| ***Jatropha* « non toxique »** | 0,01 | 2,6 | 3,4 | 9,3 | 51,0 |
| **Soja** | - | 0,4 | 4,7 | 1,5 | 12,5 |

Sources : Aderibigbe et al. (1997), Makkar et al. (1998a), Makkar et al. (1998b).

**Tableau 1.** Comparaison des teneurs moyennes en esters de phorbol dans l'amande déshuilée *Jatropha curcas* L. dits « toxiques » et « non toxiques » et des teneurs en différents composés toxiques des tourteaux de *Jatropha curcas* L. et de soja – *Comparison of mean level of phorbol esters in defatted Jatropha curcas L. kernel so-called « toxic » and « non toxic » and different levels of toxic compounds from Jatropha curcas L. and soybean meal.*

La curcine est ici exprimée en activité lectinique et correspond à la quantité minimale d'échantillon de matière nécessaire pour permettre l'agglutination d'érythrocytes dans la dilution de 1 ml d'un milieu d'essai.

### 1.3. Les terpènes et esters de phorbol

Les terpénoïdes ou terpènes sont des polymères d'une unité à cinq atomes de carbone ayant pour base un diène conjugué dont le nom commun est isoprène (2-méthylbuta-1,3-diène) (Garret et al., 2000). Ils sont formés par un assemblage progressif d'unités isoprènes (en $C_5$). Chaque unité isoprène (**Figure 1**) a « une tête », l'atome de carbone 1 et « une queue », l'atome de carbone 4 (Johnson, 2003).

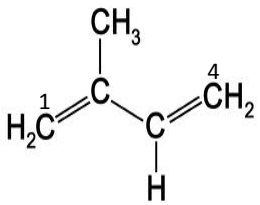


**Figure 1.** Isoprène (2-méthylbuta-1,3-diène) - *Isoprene*

Les terpènes sont des substances généralement lipophiles qui ont souvent une fonction de défense contre les insectes et les herbivores (Vollhardt et al., 2004). Tous les terpènes et leurs dérivés sont issus de la voie de biosynthèse de l'acide mévalonique (Hopkins, 2003) dont le précurseur est l'acétyl-coenzyme A (acétyl-CoA), intermédiaire du catabolisme respiratoire des glucides et des acides gras. Parmi les terpènes, les diterpènes sont les plus fréquemment rencontrés. Chez les *Euphorbiaceae* et les *Thymelaeaceae*, ces composés sont souvent très irritants pour la peau et sont toxiques non seulement pour les mammifères, mais aussi pour les phytoparasites (Marshall et al., 1985). Par ailleurs, certains diterpènes sont promoteurs de tumeurs ou co-cancérogènes. Ils favorisent la croissance des tumeurs après une exposition à une dose sub-carcinogénique d'un agent cancérigène, mais ne sont ni mutagènes ni cancérogènes (Hecker, 1987). Paradoxalement, beaucoup de ces composés ont démontré des effets anti-tumoraux. Ainsi, le taxol (paclitaxel) est un diterpène complexe, isolé de l'écorce des troncs d'if du Pacifique (*Taxus brevifolia*) qui s'est avéré être un produit très efficace contre le développement des tumeurs cancéreuses chez l'homme (Vollhart et al., 2004).

Chez les plantes, il existe des diterpènes polyhydroxylés. Rassemblés sous le nom de tiglianes (**Figure 2**), ils constituent un groupe de composés tétracycliques dont les cycles sont désignés par les lettres A, B, C et D (Haas et al., 2000). Le phorbol (**Figure 3**) fait partie des tiglianes et possède cinq groupes hydroxyles pouvant subir des réactions d'estérification. On distingue deux types de phorbol (α et β) (**Figure 4**) qui diffèrent dans leur groupement -OH au niveau du cycle C. Ce groupement rend le phorbol actif (β) ou inactif (α) selon le positionnement spatial du cycle D. Dans le phorbol β, le groupement -OH est en saillie vers le lecteur, au contraire de la position α (Goel et al., 2007). Le phorbol inactif présente la même lipophilie et les mêmes propriétés physico-chimiques que le phorbol actif, mais il est incapable de produire une réponse biologique à cause du changement conformationnel (Silinsky et al., 2003).

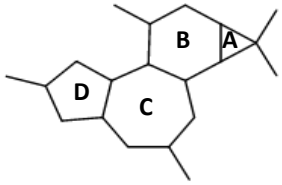


**Figure 2.** Squelette Tigliane – *Tigliane skeleton*

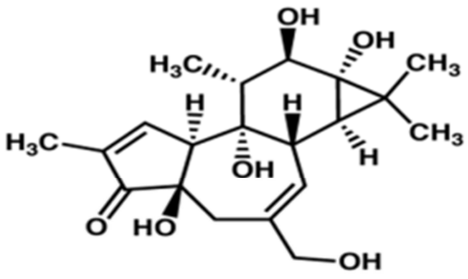


**Figure 3.** Phorbol

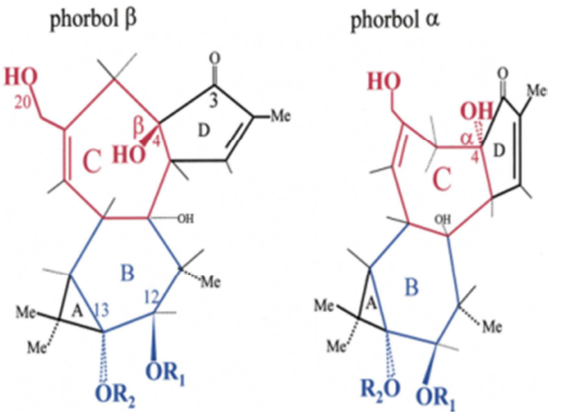


**Figure 4.** Forme des esters de phorbol – *Forms of phorbol esters*

Le phorbol donne, par condensation à diverses molécules acides, des esters dont les plus répandus sont le TPA (4β-12-*O*-tétradécanoylephorbol-13-acétate) (**Figure 5**) et le PDBu (4β-phorbol-12,13-dibutyrate) (**Figure 6**). Le TPA est également dénommé PMA (phorbol 12-myristate-13-acétate) (Silinsky et al., 2003). Ce composé se caractérise par une estérification en $C_{12}$ avec l'acide tétradécanoïque ou acide myristique [$CH_3(CH_2)_{12}COOH$] et en $C_{13}$ avec l'acide acétique ($CH_3COOH$). Le PDBu, quant à lui, se caractérise par une estérification en $C_{12}$ et $C_{13}$ avec l'acide butyrique [$CH_3(CH_2)_2COOH$]. Hirota et al. (1988) ont également mis en évidence un nouveau type d'ester de phorbol possédant une structure macrocyclique diester d'acide carboxylique. Isolé à partir de l'huile des graines de *J. curcas*, il s'agit du DHPB (12-déoxy-16-hydroxyphorbol-4′-[12′,14′-butadienyl]-6′-[16′,18′,20′-nonatrienyl]-bicyclo[3.1.0]hexane-(13-*O*)-2′-[carboxylate]-(16-*O*)-3′-[8′-acide butanoïque-10′]ate ; $C_{44}H_{54}O_8$). C'est un ester du 12-déoxy-16-hydroxyphorbol dont la partie carboxylique est une structure en anneau bicyclique qui présente une liaison en $O_{13}$ et $O_{16}$ (**Figure 7**). En outre, Haas et al. (2002) ont montré que les graines de *J. curcas* renfermaient cinq autres esters de phorbol dont la structure de base est celle du 12-déoxy-16-hydroxyphorbol. Ils se différencient par l'apparition d'unité hexane bicyclo[3.1.0] ou d'unité cyclobutane au niveau des fragments d'acide dicarboxylique. En général, il s'agit de molécules solubles dans l'acétone, le diméthylsulfoxyde, l'acétate d'éthyle, l'éthanol ou encore le chlorure de méthylène. Par contre, elles sont pratiquement insolubles dans l'eau et sont sensibles à des conditions acides et alcalines. Leur point de fusion est d'environ 72°C. Les esters de phorbol sont présents dans l'amande des graines à des concentrations variant de 0,1 à 0,7%

de la MS (Makkar et al., 2009a), alors que dans certaines variétés de *Jatropha*, notamment mexicaines, leur concentration est négligeable (0,01% de la MS).

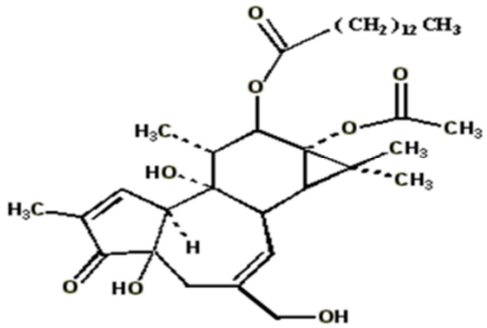

**Figure 5.** 4β-12-*O*-tétradécanoyl-13-acétate de phorbol – *4β-12-O-tetradecanoylphorbol-13-acétate*.

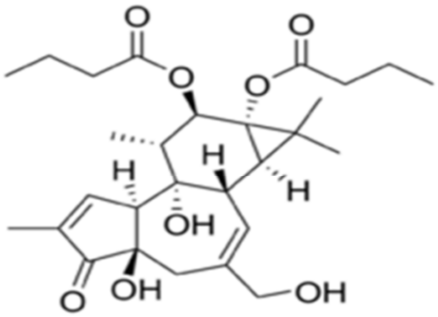

**Figure 6.** 4β-12,13-dibutyrate de phorbol – *4β-phorbol-12,13-dibutyrate*.

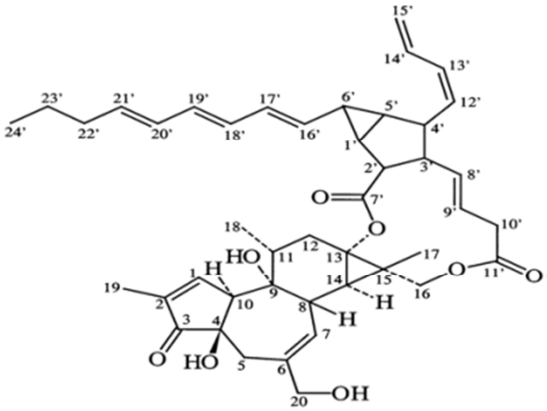

**Figure 7.** DHPB

### 1.3.1. Toxicité

La toxicité alimentaire des graines de *Jatropha* a souvent été signalée chez les animaux. Ainsi, Ahmed et al. (1979), chez des veaux nourris par sonde intragastrique à doses croissantes de poudre de graines de *J. curcas* mélangée à de l'eau, ont constaté une forte mortalité associée à des atteintes hépatiques,

digestives, rénales et pulmonaires chez les animaux qui ont reçu la concentration d'au moins 0,25 $g.kg^{-1}$. Ils confirment les observations d'Adam et al. (1975) sur des chèvres nourries aux mêmes doses de graines de jatropha et qui ont présenté des symptômes similaires suivis de mortalité. Marshall et al. (1985) ont montré une activité insecticide des esters de phorbol sur une espèce de lépidoptère parasite du cotonnier, avec une concentration nutritive de 3 $\mu g.g^{-1}$ du 12-*O*-tétradécanoylephorbol-13-acétate dans un milieu alimentaire artificiel qui a inhibé la croissance de 50% des parasites et entraîné un effet létal absolu à 20 $\mu g.g^{-1}$. Becker et al. (1998) ont constaté que des carpes rejettent des aliments contenant des esters de phorbol à partir d'une incorporation à 31 mg d'esters de phorbol par kg d'aliment. Auparavant, Gandhi et al. (1995) ont rapporté qu'à la toxicité létale par voie orale (à partir de 9 ml d'huile de *Jatropha* par kg de poids vif chez le rat) s'ajoute celle par contact. En effet, chez le lapin, une application cutanée occasionne une forte réaction inflammatoire avec œdème, érythème et nécrose, 4 h après l'application de la fraction toxique, isolée par solvants. Enfin, Li et al. (2010) ont observé que chez des souris, l'administration par voie intra-gastrique d'esters de phorbol entrainait une gastro-entérite hémorragique aigüe, avec une dose létale médiane à 27,34 $mg.kg^{-1}$ de poids vif.

Les esters de phorbol agissent de manière préférentielle sur les membranes biologiques (**Figure 8**). Ils s'intercalent dans la membrane cellulaire par le biais de récepteurs dont l'occupation active la protéine kinase C (PKC) (Blumberg et al., 1987). La PKC est présente dans tous les tissus, des mammifères aux insectes, en concentration importante dans les tissus neuronaux. Cette enzyme joue un rôle essentiel dans la transduction du signal qui régule la croissance et la différenciation cellulaire. Elle phosphoryle spécifiquement les résidus sérine et thréonine des protéines, leur permettant de réguler et de coordonner différentes voies métaboliques cellulaires (Aitken, 1987). L'activateur naturel de la PKC est le diacylglycérol dont la synthèse dépend d'une phosphodiestérase. Cette enzyme, sous l'influence de divers agonistes extracellulaires, hydrolyse des phospholipides membranaires, en particulier le phosphatidylinositol, en diacylglycérol intégré à la membrane plasmique et en inositol triphosphate qui diffuse dans le cytoplasme cellulaire. Ce dernier est responsable du déclenchement de la libération de calcium à partir des réserves internes, tandis que le diacylglycérol sert de précurseur à la production d'acide arachidonique nécessaire pour la synthèse des prostaglandines, des leucotriènes et du thromboxane et active également les PKC. Lors de l'activation, les PKC sont transportées vers la

membrane plasmique pour lui permettre de jouer son rôle dans la transduction des signaux moléculaires et donc de donner une réponse biologique (Goel et al., 2007). Les esters de phorbol, qui sont les molécules amphiphiles, ont tendance à se lier préférentiellement aux récepteurs phospholipides de la membrane cellulaire et vont agir sur les PKC de la même manière que le diacylglycérol. Ainsi, étant donné que les esters de phorbol peuvent être intercalés dans la bicouche phospholipidique de la membrane cellulaire où ils activent la protéine kinase C, en substituant le diacylglycérol, le complexe protéine kinase C – phospholipide pouvait constituer un récepteur des esters de phorbol (Kikkawa et al., 1983).

Il s'ensuit ainsi une activation importante de cette enzyme, aussi bien au niveau cytoplasmique que membranaire, lui permettant d'agir notamment dans la médiation de la réponse cellulaire aux stimuli extracellulaires conduisant en particulier à la prolifération et à la différenciation cellulaire ou à l'apoptose. Ces phénomènes se traduisent principalement par une inflammation ou une progression tumorale (Goel et al., 2007). D'ailleurs, chez la souris, Evans et al. (1987) ont montré que le meilleur test pour évaluer l'activité pro-inflammatoire de l'ester de phorbol est la détermination de l'érythème sur la peau. L'apparition de l'érythème ne semble pas due à un effet direct des esters de phorbol sur la peau, mais à la libération, sous l'effet des esters de phorbol, de médiateurs endogènes de l'inflammation au niveau de la micro vascularisation. Par ailleurs, Hecker (1987) a montré que, dans une population à risque, une inflammation chronique causée par les esters de phorbol peut entrainer l'activation de virus oncogènes pouvant participer à un éventuel développement tumoral.

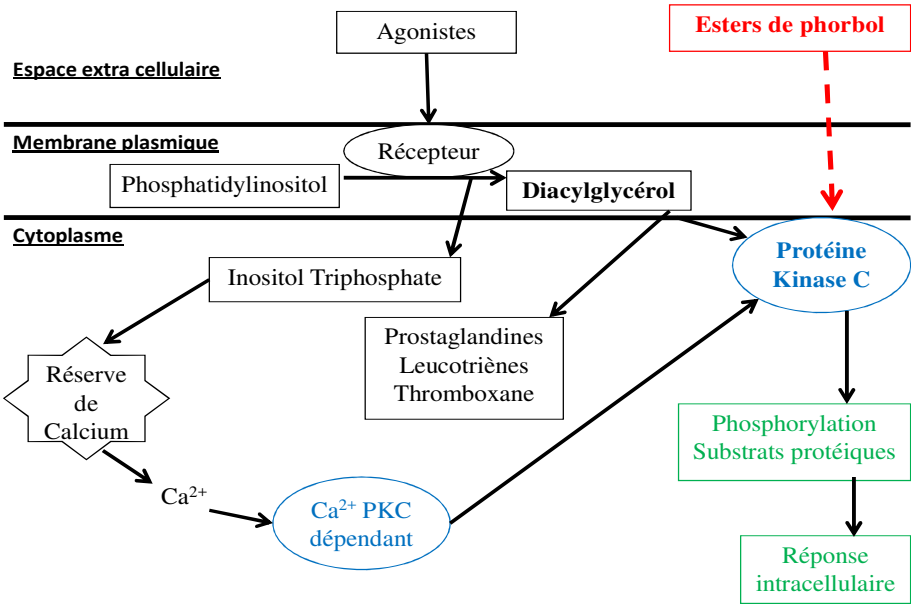


**Figure 8.** Activation de la protéine kinase C et rôle des esters de phorbol – *Activation of protein kinase C and role of phorbol esters*

### 1.3.2. Procédés de mise en évidence des esters de phorbol

Au cours des procédés d'isolement, les esters de phorbol naturels sont relativement instables et sensibles à des réactions d'oxydation, d'hydrolyse, de transestérification et d'épimérisation (Haas et al., 2002). Leur isolement doit donc être réalisé en milieu anaérobie. Aussi, les solvants d'extraction doivent être dégazés et l'extraction effectuée sous un flux continu d'azote ou d'argon. Les protocoles d'isolement se basent sur l'estérification des groupes fonctionnels hydroxyles des esters de phorbol par des agents chimiques. Les esters de phorbol ainsi dérivés sont séparés par des méthodes de chromatographie en phase liquide haute performance (*High Performance Liquid Chromatography* – HPLC). L'extrait contenant les esters de phorbol peut être purifié en utilisant la chromatographie sur couche mince (*Thin-Layer Chromatography* – TLC), tandis que la structure du composé obtenu est déterminée par spectrométrie de masse (Goel et al., 2007).

Pour quantifier les esters de phorbol, Makkar et al. (1997) ont broyé des graines de *J. curcas* dans du dichlorométhane en présence de sable abrasif. Le filtrat obtenu a été soumis aux ultra-sons et le résidu a été séché sous vide, puis dissout dans du tétrahydrofurane et filtré avant d'être analysé par HPLC. La phase stationnaire de la colonne analytique et de la pré-colonne était une phase inverse $C_{18}$. Les phases mobiles utilisées en mode gradient comportaient de l'acide *O*-phosphorique, de l'acétonitrile et du

tétrahydrofurane, tous dégazés par ultrasons. En réalisant la séparation à température ambiante (22°C) et à un débit de 1,3 ml.min$^{-1}$, les esters de phorbol (de quatre types) sont élués entre 41 et 48 min et détectés à 280 nm. Martínez-Herrera et al. (2006) ont obtenu une séparation satisfaisante à température ambiante, avec une même colonne analytique (250 x 4 mm), un même débit de 1,3 ml.min$^{-1}$ en utilisant un gradient d'élution composé d'un mélange d'acétonitrile et d'eau, après une extraction au méthanol suivie d'une centrifugation. Les pics d'esters de phorbol apparaissent dans ces conditions entre 26 et 31 min et sont détectés à 280 nm. Dans cette approche, les résultats ont été exprimés en phorbol-12-myristate 13-acétate (apparu entre 34 et 36 min).

### 1.4. Les protéines

#### 1.4.1. La curcine

Certaines protéines, appelées protéines de stockage, sont produites par les plantes et accumulées de manière à constituer des réserves d'acides aminés et d'énergie, qui sont mobilisées en particulier lors du processus de germination des graines. Parmi ces protéines, les lectines (Kaufmann et al., 1999) ou hémagglutinines peuvent constituer souvent 6 à 11% des protéines totales des graines de nombreuses plantes. Chez les végétaux, elles se rencontrent essentiellement chez les *Fabaceae* (légumineuses) et les *Euphorbiaceae*, mais on les trouve aussi chez les champignons, les bactéries et les animaux. Elles peuvent constituer pour la plante un moyen de repousser les herbivores, grâce à leur affinité pour les glycoprotéines membranaires de la muqueuse digestive, entrainant des troubles gastro-intestinaux. Plus généralement, leur capacité à se lier à des sucres les fait intervenir dans les phénomènes de reconnaissance de symbiotes, de pathogènes, mais aussi de grains de pollen. Par ailleurs, elles ont la propriété d'agglutiner spécifiquement les hématies (Hopkins, 2003). Ainsi, leur présence est mise en évidence par des réactions de précipitation avec des oligosaccharides, par exemple des particules de latex recouvertes de résidus de sucre (Kaul et al., 1991) et des réactions d'agglutination de cellules végétales et animales, telles que les éryhtrocytes (Gordon et al., 1974).

La curcine ou curcasine est une lectine produite par *J. curcas*. Cette toxalbumine est proche de la ricine produite par *Ricinus communis* et de la crotine produite par *Croton tiglium* (Seigler, 1998), plantes appartenant toutes à la famille des *Euphorbiaceae*. Bien que toutes les parties de la plante contiennent de la curcine, l'amande des graines présente les plus fortes concentrations, mais la coque

en est dépourvue. Les propriétés irritantes des graines de *J. curcas*, de la variété toxique, en particulier pour les personnes qui les manipulent, sont généralement attribuées à la présence de curcine. Cependant, les teneurs en curcine étant similaires entre les variétés de jatropha non toxiques et toxiques, il semble que ce composé ne soit pas le principal élément toxique des graines de *Jatropha* (Makkar et al., 1998a). La curcine est une glycoprotéine qui présente une masse moléculaire d'environ 28,2 kDa (kilodalton) avec des teneurs en sucres d'environ 4,91% et 251 acides aminés constitués de 31 Asp., 15 Thr, 16 Ser., 22 Glu., 9 Pro., 15 Gly., 22 Ala., 26 Val., 2 Mét., 14 Ile., 24 Leu., 14 Tyr., 12 Phé., 18 Lys., 2 His., 7 Arg., 1 Cys. et 1 Trp. (Lin et al., 2003 ; Lin et al., 2010).

Dans la plante, la curcine joue un rôle défensif et confère une immunité contre de nombreux ravageurs. Stirpe et al. (2006) ont montré que la curcine présente une activité inhibitrice contre certains virus de plantes, de champignons et d'animaux, permettant une application possible dans l'agriculture.

La curcine et la ricine font partie d'une famille très diversifiée de « protéines inactivant les ribosomes » (*ribosome inactivating protein – RIP*) qui comprennent de nombreuses toxines bactériennes ou de plantes supérieures. Ces inhibiteurs de la synthèse protéique agissent en inactivant des sous-unités ribosomales (Millard et al., 2008). On distingue trois types d'inhibiteurs de la synthèse protéique. Les *RIP* de type 1, auxquelles appartient la curcine, sont des N-glycosidases monomériques à faible capacité de liaison cellulaire ou d'internalisation. Par contre, les *RIP* de type 2, notamment la ricine, sont composées de deux sous-unités structurelles reliées par un pont disulfure, une chaine A de nature enzymatique, la N-glycosidase, et une chaine B qui a des sites de fixation où adhèrent des sucres ayant la configuration du D-galactose (Stirpe et al., 1986). Cette dernière se lie à un glycane à la surface cellulaire et facilite l'endocytose, rendant ainsi ces *RIP* particulièrement toxiques. Enfin, les *RIP* de type 3, moins connues, sont des précurseurs inactifs de N-glycosidase, moins communs.

La curcine inhibe la traduction de l'ARN messager en chaînes polypeptidiques extracellulaires en présentant une activité N-glycosidase sur le fragment ribosomal de l'ARN similaire à celle de la ricine avec les mêmes résidus d'acides aminés formant le site actif de la chaîne A de la ricine à l'exception de la Gln-173 remplacée par la Glu-163 dans la curcine (Lin et al., 2003).

Ainsi, la curcine inhibe la synthèse des protéines *in vitro*, de manière comparable à la ricine, mais elle se révèle 1 000 fois moins toxique en raison de son incapacité à pénétrer les cellules. Cependant, à l'inverse de beaucoup de *RIP* de type 1, elle contient un résidu de cystéine qui peut former un pont disulfure avec un anticorps activé conférant à celui-ci une cytotoxicité maximale (Luo et al., 2007). Il faut noter que l'on retrouve les *RIP* de type 1 dans de nombreuses matières végétales comestibles, comme des grains de céréales, les betteraves, les épinards, rendant ainsi peu probable le fait que la présence de curcine dans les graines de *J. curcas* puisse être un obstacle à leur valorisation en alimentation animale. La production de curcine est favorisée par des situations de stress pour la plante aussi bien abiotiques que biotiques (Qin et al., 2005) avec une faible activité au sein de la plante pouvant être dûe soit à une faible concentration, soit à une faible sensibilité des ribosomes végétaux et bactériens par rapport aux ribosomes des mammifères (Stirpe et al., 1986 ; Girbés et al., 2004).

### 1.4.2. L'inhibiteur de l'activité de trypsine

Selon Ryan (1990), les plantes comme *J. curcas* ont développé des stratégies de lutte passive ou des mécanismes de résistance induite. La production de protéines de défense comme les inhibiteurs de protéases, réduisant la digestibilité des tissus de la plante pour certaines espèces animales, fait partie de la seconde catégorie. Il s'agit en général de protéines qui forment des complexes avec les protéases permettant d'inhiber leur activité protéolytique. On en trouve souvent dans les tissus et les liquides qui sont vulnérables à l'action de protéases : le sérum sanguin, les cellules acineuses pancréatiques, mais aussi les tissus de stockage des plantes, en particulier les graines. La présence d'inhibiteurs en forte concentration dans les aliments peut modifier de manière significative les processus de digestion et interférer avec la croissance et le développement de l'animal. Chez les monogastriques nourris avec des tourteaux contenant de tels inhibiteurs, l'inactivité enzymatique résultant de la formation des complexes avec la trypsine et la chymotrypsine entraine une hypersécrétion d'enzymes pancréatiques (Rackis et al., 1981). Une stimulation chronique conduit à l'hypertrophie du pancréas et ainsi une inhibition de la croissance des jeunes animaux par perte fécale excessive de protéines non digérées.

## 1.5. Les saponines

Les saponines sont une famille diversifiée de métabolites secondaires qui présentent une action thérapeutique et qui produits par de nombreuses espèces de plantes dont le plupart sont utilisées en

médecine traditionelle (Sparg et al., 2004). Ce sont des triterpènes glycosidiques (**Figure 9**) de haut poids moléculaire formés d'une partie aglycone, la sapogénine – triperpenoïde ou stéroïde – liée avec une partie glycosidique – chaine latérale osidique (glucose, galactose, acide glucoronique, xylose, rhamnose ou méthylose) (Seigler, 1998).

Toutes les saponines ont en commun l'attachement d'une ou plusieurs chaînes de sucre avec l'aglycone permettant de distinguer les saponines mono, bi ou tridesmoside selon la fixation de la molécule à une, deux ou plus rarement trois chaînes de sucres. Selon la nature de la sapogénine, les saponines peuvent être divisées en trois catégories principales (Hostettmann et al., 1995 ; Bruneton, 2008) :

- Les saponines à génine triterpénique qui sont les plus courantes, présentes dans les angiospermes dicotylédones et dont les triterpènes pentacycliques peuvent être divisés en trois catégories principales, selon qu'ils ont un squelette β-amyrine (oléanane), α-amyrine (ursane) ou lupéol.
- Les saponines à génine stéroïde dont la plupart sont dérivées du squelette furostan ou spirostan et presque exclusivement présents dans les angiospermes monocotylédones.
- Les saponines à génine alcaloïde stéroïdien constitués de deux classes, les solanidans et les spirosolans.

Largement distribuées dans le règne végétal, elles ont des propriétés détergentes et forment facilement des mousses en solution aqueuse. Historiquement, de nombreuses plantes contenant des saponines sont utilisées comme savons. Les saponines sont amères et se révèlent être très toxiques, notamment pour les poissons, par leur activité hémolytique (Kaufmann et al., 1999). Aderibigbe et al. (1997) ont mis en évidence moins de saponines (en moyenne 2,3% de la MS) dans les tourteaux d'amande de *J. curcas* que dans le tourteau de soja (4,7% de la MS) ; dans ce dernier toutefois, les saponines sont non hémolytiques et inoffensives pour la consommation animale (Liener, 1979).

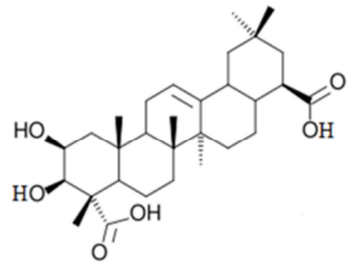


**Figure 9.** Saponine triterpénoïde (acide médicagénique) – *Triterpenoid saponin (medicagenic acid)*

### 1.6. Le phytate

L'acide phytique, encore appelé acide myo-inositol hexaphosphorique, est le constituant phosphoré le plus abondant des végétaux (**Figure 10**). Il constitue la principale forme de stockage du phosphore dans la fibre des graines de céréales, des graines de légumineuses et des noix (Spiller & Spiller, 2005). Il se trouve notamment dans les graines de *Jatropha curcas*, où il est associé à une enzyme, la phytase, dont le rôle consiste à le décomposer en unités d'inositol et de phosphate lorsque la graine commence à germer. Il représente un facteur antinutritionnel pour les animaux en liant les éléments minéraux tels que le calcium, le zinc ou le fer, formant ainsi des complexes insolubles dénommés phytates qui limitent l'absorption de ces mêmes éléments minéraux au niveau du tractus digestif (Cheryan et al., 1980).

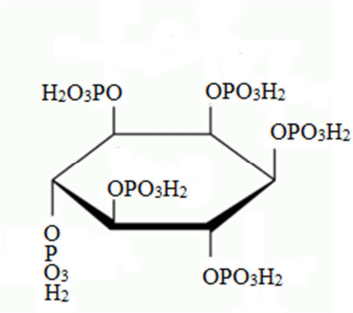


**Figure 10** : Acide phytique ($C_6H_{18}O_{24}P_6$) – *Phytic acid* (source Frénot & Vierling, 2001)

On trouve dans les tourteaux de jatropha, aussi bien dans les variétés de la plante toxique que non-toxique, des quantités très élevées d'acide phytique (environ 9% par rapport à la matière sèche) (Makkar et al., 2009) comparativement à celles contenues dans le tourteau de soja (Makkar et al., 1998a) qui sont pourtant considérées comme étant élevées. Ces phytates vont non seulement réduire la biodisponibilité des minéraux chez les monograstiques (Erdman, 1979), mais sont aussi impliqués

dans la réduction de la digestibilité des protéines en formant des complexes et en interagissant avec des enzymes telles que la tryspine et la pepsine (Reddy & Pierson, 1994). Leur teneur n'est pas influencée par les traitements thermiques (Aderibigbe et al., 1997), notamment la cuisson à la chaleur sèche (Makkar et al., 1998b) mais bien par des phytases biologiques ou de synthèse (Simons et al., 1990 ; Jongbloed et al., 1992 ; Martínez-Herrera et al., 2006). La phytase, connu sous le nom de myo-inositol hexakisphosphate phosphohydroxylase va catalyser l'hydrolyse du phytate, permettant de rendre le phosphore disponible pour absorption (Ahmad et al., 2000 ; Kumar et al., 2011). Une alternative consiste à complémenter la ration alimentaire avec un prémélange de vitamines et minéraux pour améliorer l'utilisation nutritive du phosphore et du magnésium (Porres et al., 2004).

### 1.7. Procédés de détoxification des tourteaux de graines de jatropha

En raison de la présence de composés toxiques et de facteurs antinutritionnels dans les graines de certaines variétés de *J. curcas*, l'huile mais aussi les tourteaux issus de l'extraction de l'huile ne peuvent être valorisés tels quels. Une détoxification totale est généralement nécessaire pour la plupart des applications, en particulier la nutrition. Diverses méthodes physiques, chimiques ou biologiques de détoxification partielle sont rapportées dans la littérature, selon le mode d'extraction de l'huile. Lorsque la détoxification est réalisée par voie mécanique (trituration), l'ester de phorbol se répartit en moyenne à raison de 70% dans l'huile et de 30% dans les tourteaux (Makkar et al., 2009a). Si Aregheore et al. (2003) ont montré que des traitements thermiques (121°C pendant 30 min) ne modifient pas la concentration en esters de phorbol dans les tourteaux de jatropha, l'inactivation de la curcine peut être réalisée par un traitement à la chaleur humide : 80% d'humidité, à 130°C, pendant 30 min (Aregheore et al., 1998 ; Chivandi et al., 2004). Cela pourrait être lié à une perte d'acides aminés impliquant leur faible disponibilité en raison d'une réaction de Maillard qui a entraîné un changement dans la structure des protéines impliquées (Makkar et al., 1999), ce qui n'est pas le cas des esters de phorbol.

De même, les inhibiteurs de la trypsine peuvent être facilement éliminés par la cuisson ordinaire et le traitement à la chaleur humide. Makkar et al. (1998b) ont également montré que des graines de jatropha grillées pendant environ 15 min deviennent exemptes de facteurs antitrypsiques. Martínez-Herrera et al. (2006) ont confirmé que ces facteurs peuvent être inactivés à plus de 98% par un

traitement thermique à 121°C pendant 25 min environ. Par contre, les saponines sont des molécules thermostables et sont considérées comme des facteurs antinutritionnels, surtout pour les monogastriques. Leur ingestion ne provoque en général que des retards de croissance et une valeur énergétique réduite de la ration, sans mortalité.

Le mode d'extraction de l'huile présente une incidence non négligeable sur les teneurs en esters de phorbol. L'extraction par solvant permet d'extraire 99% de l'huile, ce qui n'est pas le cas avec une extraction par presse, en mode artisanal (Beerens, 2007).

Haas et al. (2000) ont tenté, par voie chimique, de réduire les teneurs en esters de phorbol dans l'huile de *J. curcas*. Le raffinage de l'huile par dégommage (élimination des phosphatides) à l'aide d'acide phosphorique, suivi de neutralisation à l'hydroxyde de sodium et Désodorisation qui s'est faite par distillation sous vide poussé à une température d'environ 200°C pendant 2 heures. Le but étant d'éliminer acides gras et les substances odoriférantes, notamment les aldéhydes et les cétones, par entraînement à la vapeur sous vide et à température élevée. Cette désodorisation n'a réduit la teneur en esters de phorbol que d'environ 50%. Cependant, un effet destructeur sur les esters de phorbol a été observé lors de la désodorisation d'échantillons d'huile à des températures élevées (260°C) en présence d'hydroxyde de sodium (Makkar et al., 2009b). Le méthanol d'une part, et l'hydroxyde de sodium combiné à l'hypochlorite de sodium d'autre part, se sont révélés efficaces pour détruire les esters de phorbol. Aregheore et al. (2003) ont, grâce à ces deux traitements, réduit la concentration en esters de phorbol de respectivement 95 et 93%. Ces résultats ont été confirmés par Ahmed et al. (2009) qui ont montré que l'extraction des esters de phorbol avec le méthanol permettait de diminuer en moyenne le niveau d'esters de phorbol dans l'huile de jatropha de 85%. Devappa et al. (2010a), quant à eux, ont réduit d'environ 80% les teneurs en esters de phorbol de l'huile après dilution au méthanol, évaporation et raffinage. Plus polaires que les principaux lipides présents dans les tourteaux de jatropha, les esters de phorbol ne peuvent être extraits efficacement par des solvants apolaires tels que l'hexane. Chivandi et al. (2004) ont appliqué, à des amandes broyées, un traitement industriel impliquant d'une part, une double extraction au solvant (hexane-éthanol) accompagnée d'une phase extrusion-traitement à la vapeur et d'autre part, le même traitement suivi d'une nouvelle extraction à l'hexane ainsi qu'une extrusion par voie humide. Ils ont ainsi réduit les teneurs en esters de phorbol de

respectivement 71 et 88%. Enfin, Rakshit et al. (2008) avaient observé qu'après extraction à l'hexane, un traitement à l'hydroxyde de sodium permettait de réduire les teneurs en esters de phorbol de 88 et 89% pour respectivement les graines décortiquées et les graines non décortiquées, contre 90 et 81% respectivement avec l'hydroxyde de calcium. Par ailleurs, Martínez-Herrera et al. (2006) ont montré que sur des tourteaux décortiqués obtenus par solvant, une extraction à l'éthanol, avec ou sans autoclavage, permettait de réduire les teneurs en esters de phorbol de plus de 95%, contre 18% avec un simple traitement au bicarbonate de sodium. Ils ont ainsi confirmé la forte affinité des esters de phorbol avec les alcools les plus simples, modérément polaires. L'utilisation de l'éthanol présente, selon eux, l'avantage de ne laisser aucun résidu toxique dans les tourteaux. Cette même extraction à l'éthanol permettrait, en outre, de baisser la teneur en saponines de ces tourteaux d'environ 50% et, suivie d'un traitement au bicarbonate de sodium puis d'un chauffage à 121°C pendant 30 min, elle permettrait également de diminuer les teneurs en curcine d'environ 94%. Concernant les saponines, Abou-Arab et al. (2010) sont parvenus à des résultats similaires, ou meilleurs (soit 51,33% de réduction), sur des amandes des graines de jatropha délipidées en combinant traitements chimiques (éthanol et bicarbonate de sodium) et thermiques.

### 1.8. Procédés de détoxification biologique du tourteau de *Jatropha curcas* et utilisation potentielle en alimentation animale

Malgré la teneur en nutriments, notamment en protéines, l'utilisation du tourteau de jatropha en alimentation des animaux est restée marginale. Des procédés de détoxification permettant l'amélioration de son emploi pourraient amener ainsi un changement important dans l'économie agricole en fournissant une source de revenu supplémentaire pour les agriculteurs. Différents procédés de détoxification biologique ont été décrits dans la littérature venant compléter des procédés physiques et chimiques, permettant ainsi une utilisation des tourteaux en nutrition animale.

En effet, les traitements thermiques par chaleur humide (67% à 100°C pendant 30 mn ou 80% à 130°C pendant 30 mn) ont permis d'inactiver la curcine, mais aussi les inhibiteurs de la trysine pour des tourteaux dégraissés (Aderibigbe et al., 1997 ; Aregheore et al., 1998 ; Lajolo et al., 2002). Les phytates, les saponines et les esters de phorbol, thermostables, ne sont pas affectés par ces traitements.

Des traitements chimiques ont permis, quant à eux, de réduire les esters de phorbol qui constituent le facteur toxique le plus important du tourteau de jatropha. Ainsi, des extractions successives avec des solvants de polarité différente (hexane puis méthanol) (Gaur, 2009) ont permis de réduire les teneurs en esters de phorbol (0,06 contre 0,11 mg/g de tourteau). Les esters de phorbol, qui sont plus polaires que les principaux lipides présents dans les tourteaux de jatropha, ne pouvaient être efficacement extraits par l'hexane, qui permet surtout l'extraction les matières grasses peu polaires mais l'étaient par le méthanol qui est un solvant polaire. Ces résultats ont été confirmés car, suite à des rinçages successifs au méthanol à 92% ou à une extraction avec l'éthanol à 90% pendant 2 heures, il a été possible d'éliminer, respectivement, 94,9 et 95,8% des esters de phorbol présents dans des tourteaux de jatropha (Aregheore et al., 2003 ; Martinez-Herrera et al., 2006).

Les traitements chimiques combinés aux traitements thermiques ont aussi donné de bons résultats. Les esters de phorbol ont pu, dans ce cas, être réduits de 97,9% grâce à des extractions avec de l'éthanol, suivis d'un mélange au bicarbonate de sodium à 0,07% puis d'un autoclavage à 121°C pendant 20 mn (Martinez-Herrera et al., 2006). Cela a permis une réduction de l'activité lectinique ainsi que des inhibiteurs de la trypsine, sensibles à la chaleur.

Pour compléter les traitements physiques et chimiques, les traitements biologiques se sont révélés efficaces et prometteurs pour détoxiquer les tourteaux de jatropha dans une approche écologiquement rationnelle et économiquement avantageuse. Ainsi, à partir du cytosol de foie de souris, a été isolée une estérase hydrophobe qui hydrolyse exclusivement le diester 12-O-tétradécanoylphorbol-13-acétate (TPA) actif en un monoester tétradécanoylphorbol-13-acétate inactif au niveau des voies métaboliques de promotion des tumeurs (Shoyab et al., 1981). Ces observations ont été confirmées par la mise en évidence d'une estérase 1, présente dans la plupart des tissus de souris, capable de rompre les esters d'acides gras en ayant une activité phorbol-12-ester hydrolase (Kadner et al., 1985). En outre, une carboxylestérase non spécifique présente dans le réticulum endoplasmique du foie de rat a été identifiée (Mentlein, 1986). Elle s'est avérée capable de convertir, *in vitro*, le TPA en un monoester phorbol 13-acétate, et donc de détoxiquer ce promoteur de tumeur. Ce type de détoxication est une approche environnementale saine pour faire de *J. curcas* un aliment approprié pour les animaux.

Par fermentation du tourteau de *J. curcas* avec des champignons, notamment *Aspergillus niger* (Belewu et al., 2011a), les esters de phorbol ont été réduits de 76,9%. Cette fermentation a aussi permis de réduire, respectivement, de 92,4 ; 68,3 ; 94,7 et 70,3% les substances anti nutritionnelles telles que la curcine, les inhibiteurs de la trypsine, les saponines et les phytates. Grâce aux mêmes souches *d'Aspergillus niger*, 92% de la caféine a été dégradée, ainsi que des tanins présents dans la pulpe de café utilisée comme substrat de fermentation pour ces champignons et permettant ainsi son utilisation en alimentation animale (Brand et al., 2000).

La substitution partielle du tourteau de soja par du tourteau de jatropha ensemencé avec divers champignons (*Aspergillus niger*, *Rhizopus oligosporus* et *Penicillium sp.*) a permis une bonne croissance de chèvres et une bonne digestibilité de la ration, sans influence sur les paramètres hématologiques et sur la viabilité des animaux (Belewu & Akande, 2010 ; Belewu & Sam, 2010 ; Belewu et al., 2010a ; Belewu et al., 2010b). Ces résultats sont confirmés par l'utilisation, outre d'*A. niger*, d'autres souches de champignons (*Penicillium chrysogenum* et *Trichoderma harzanium*) (Belewu et al., 2011b). L'aliment obtenu a, ici aussi, été bien appété par les animaux qui n'ont manifesté aucun problème de santé et l'impact a été positif sur la digestibilité des nutriments (Belewu et al., 2011c). Il s'agit d'une technologie simple à mettre en œuvre, bon marché et prometteuse pour l'alimentation des animaux, notamment celle des ruminants pendant les périodes sèches. Par ailleurs, un diterpène de type jatrophone, décrit comme cytotoxique (Devappa et al., 2010b) dont l'extrait a été soumis à une culture d'*Aspergillus niger*, a subi une biotransformation en un nouveau diterpène 9β-hydroxy isabellione. Cela a permis de réduire fortement sa toxicité évaluée sur des cellules épithéliales gastriques humaines (Pertino et al., 2007).

D'un point de vue nutritionnel, l'ensemencement d'*Aspergillus niger* sur du tourteau de jatropha permet, outre une réduction des niveaux d'esters de phorbol et des facteurs anti nutritionnels, un enrichissement en nutriments par augmentation de 7,5% des teneurs en protéines brutes notamment (Dinis et al., 2009 ; Rosa et al., 2010). La combinaison de quatre espèces de champignons (*Aspergillus niger*, *Penicillium sp.*, *Trichoderma harzanium* et *Trichoderma longibrachiatum*) sur du tourteau d'amande de graine de jatropha (Belewu et al., 2011a) permet, également, de réduire les teneurs en facteurs anti nutritionnels. En utilisant *Bjerkandera adusta* et *Phlebia rufa*, des souches de moisissures

ligninolytiques, ensemencées sur du tourteau de jatropha, la concentration en esters de phorbol a été réduite de, respectivement, 91 et 97% (De Barros et al., 2011). D'un autre côté, grâce à une souche bactérienne de *Pseudomonas aeruginosa* PseA, la concentration en esters de phorbol a été complètement réduite en 9 jours de fermentation sur du tourteau de jatropha (Joshi et al., 2011). Enfin, une baisse respective de la concentration des inhibiteurs de la trypsine et de la curcine d'environ 82 et 86,7% a été possible par un traitement bactérien des tourteaux avec *Lactobacillus acidophillus* (Abo El-Fadel et al., 2011).

Dans l'alimentation de poulets de chair, *Neurosphora sitophila* et *Aspergillus oryzae* mis en fermentation sur du tourteau de jatropha, ont permis de réduire la toxicité liée aux esters de phorbol et ainsi le taux de mortalité des volailles, mais sans impact significatif sur les performances des animaux (Wina et al., 2010).

Enfin, par hydrolyse des parois cellulaires à l'aide de cellulases et de pectinases, suivie d'un rinçage à l'éthanol, il a été possible de supprimer totalement la teneur en esters de phorbol et de réduire les facteurs antinutritionnels (Xiao et al., 2011) du tourteau de jatropha. Corrélativement, une amélioration de 25,5% de la teneur en protéines brutes et en acides aminés essentiels, mais aussi une amélioration de la digestibilité protéique *in vitro* de 12,5% s'est faite, permettant d'améliorer les qualités nutritionnelles (Abarca et al., 1994).

Parallèlement à la réduction des composés toxiques et antinutritionnels, une baisse des taux des glucides, de la matière grasse et des fibres brutes a été notée (Oseni et al., 2001). En effet, La croissance fongique s'accompagne d'une baisse du contenu hémicellulosique et d'une baisse des facteurs antinutritionnels (Elyas et al., 2002), liée à l'utilisation de ces nutriments pour le métabolisme fongique. A cela s'ajoute une amélioration de la teneur en protéines brutes des échantillons traités par les champigons liée, sans doute, à l'addition de la protéine microbienne au cours du processus de fermentattion ou encore à la synthèse de produits finaux comme celles d'enzymes extracellulaires pour dégrader les composés antinutritionnels (Belewu & Sam, 2010 ; Faoziyat et al., 2014).

La fermentation sur substrat solide est effectuée sur du matériau non soluble qui agit à la fois comme support physique et source de nutriments (Pandey, 1992 ; Raimbault, 1998). La teneur faible en humidité signifie que la fermentation ne peut être effectuée que par un nombre limité de

microorganismes, principalement les levures et les champignons, bien que certaines bactéries soient également utilisées (Pandey et al., 2000). Il s'agit d'une technologie potentielle dans la désintoxication biologique des résidus agro-industriels et la production de produits à valeur ajoutée tels que des métabolites secondaires ou des enzymes (Pandey, 2003). Les champignons peuvent être considérés comme les organismes les mieux adaptés à la fermentation sur substrat solide. Leurs hyphes, non seulement peuvent se développer en surface (Dos Santos et al., 2004), mais aussi pénétrer dans les espaces inter particulaires et ainsi coloniser le substrat. Outre la détoxication biologique des résidus de culture, cette technologie est de plus en plus utilisée pour la production de produits à valeur ajoutée tels que les métabolites secondaires. Ainsi, la fermentation en milieu solide détient un énorme potentiel pour la production de la presque totalité des enzymes microbiennes connues ; le substrat fournissant aux microorganismes nutriments et ancrage (Pandey, 1992). Parmi les enzymes produites, les pectinases, les lipases, les tannases ainsi que les phytases (Gramiha et al., 2008 ; Longo et al., 2008 ; Madeira et al., 2011) réduisent les facteurs antinutritionnels en alimentation animale. Ainsi, grâce à la fermentation d'une souche de *Penicillium simplicissimum* sur un substrat constitué de tourteau de graines de ricin, la production de lipase a été stimulée (Godoy et al., 2009). En outre, dans le cas des champignons filamenteux, l'utilisation particulière d'*A. niger* (Walker, 2002) ne provoque pas de problèmes particuliers de manipulation chez l'homme et la formation de toxine n'a pas été observée dans des conditions de fermentation contrôlée (Schuster et al., 2002 ; Wina et al. 2010).

### 1.9. Conclusion

Pour faire face à l'augmentation de la demande en matières premières alimentaires pour l'élevage, liée à une forte croissance de la population dans les pays en développement, des recherches se sont orientées vers de nouveaux types d'aliments qui ne constituent pas la base de l'alimentation humaine. Les graines de *Jatropha curcas* font partie de cette catégorie et pourraient même être considérées comme un aliment de remplacement de produits conventionnels. Outre le fait que le tourteau de ces graines pourrait être un bon substrat pour la production d'enzymes industrielles, il pourrait constituer une excellente source de protéines pour les animaux. La fermentation microbienne ou fongique sur un substrat constitué de tourteau de jatropha améliore la teneur de certains nutriments et réduit celle des

composés toxiques et anti nutritionnels. Cela rend ainsi possible leur utilisation comme matière première, aliment ou supplément protéique pour les animaux.

Ainsi, malgré des résultats encourageants obtenus avec les méthodes de détoxification physique et chimique, les méthodes biologiques se sont avérées prometteuses pour, non seulement baisser voire supprimer simultanément les composés toxiques et anti nutritionnels, mais aussi pour améliorer les qualités nutritionnelles des tourteaux de jatropha.

D'autre part, les complexes enzymatiques produits par les moisissures ligninolytiques ont un énorme potentiel dans le traitement des aliments fibreux pour améliorer leur valeur nutritive.

La biotransformation des résidus de culture en vue l'amélioration de leurs qualités nutritionnelles reste une application importante pour la fermentation sur substrat solide. Par l'utilisation de résidus agro-industriels comme les tourteaux, notamment ceux sous ou non-utilisés comme le cas particulier du *J. curcas*, les processus de fermentation en substrat solide offrent un moyen écologique de production de valeur ajoutée.

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**Références bibliographiques**

ABARCA M.L., BRAGULAT M.R., CASTELLA G. & CABANES F.J., 1994. Ochratoxin A production by strains of *Aspergillus niger* var. *niger*. *Appl. Environ. Microbiol.*, **60**(7): 2650-2652.

ABO El-FADEL M.H., HUSSEIN A.M. & MOHAMED A.H., 2011. Incorporation *Jatropha curcas* meal on lambs ration and its effect on lambs performance. *J. Am. Sci.*, **7**(2): 129-132.

ABOU-ARAB A.A. & ABU-SALEM F.M., 2010. Nutritional quality of *Jatropha curcas* seeds and effects of some physical and chemical treatments on their anti-nutritional factors. *Afr. J. Food Sci.*, **4**(3): 93-103.

ADAM S.E. & MAGZOUB M., 1975. Toxicity of *Jatropha curcas* for goats. *Toxicology*, **4**(3): 347-354.

ADERIBIGBE A.O. et al., 1997. Chemical composition and effect of heat on organic matter – and nitrogen – degradability and some antinutritional components of jatropha meal. *Anim. Feed Sci. Technol.*, **67**(2-3): 223-243.

AHMAD T. et al., 2000. Effect of microbial phytase produced from fungus *Aspergillus niger* on bioavailability of phosphorus and calcium in broiler chickens. *Anim. Feed Sci. Technol.*, **83**(2): 103-114.

AHMED O.M.M. & ADAM S.E.I., 1979. Effects of *Jatropha curcas* on calves. *Vet. Pathol.*, **16**(4): 476-482.

AHMED W.A. & SALIMON J., 2009. Phorbol ester as toxic constituents of tropical *Jatropha curcas* seed oil. *Eur. J. Sci. Res.*, **31**(3): 429-436.

AITKEN A., 1987. The activation of protein kinase C by daphnane, ingenane and tigliane diterpenoid esters. *Bot. J. Linean Soc.*, **94**(1-2): 247-263.

AREGHEORE E.M., MAKKAR H.P.S. & BECKER K., 1998. Assessement of lectin activity in a toxic and a non-toxic variety of *Jatropha curcas* using latex agglutination and haemagglutination methods and inactivation of lectin by heat treatments. *J. Sci. Food Agric.*, **77**(3): 349-352.

AREGHEORE E.M., BECKER K. & MAKKAR H.P.S., 2003. Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats. *S. Pac. J. Nat. Sci.*, **21**(1): 51-56.

BECKER K. & MAKKAR H.P.S., 1998. Effects of phorbol esters in carp (*Cyprinus carpio* L.). *Vet. Human Toxicol.*, **40**(2): 82-86.

BEERENS P., 2007. Screw-pressing of jatropha seeds for fuelling purposes in less developed countries. Eindhoven, The Netherlands: Eindhoven University of Technology, Department of Sustainable Energy Technology.

BELEWU M.A. & AKANDE B.A., 2010. Biological upgrading of the nutritional quality of *Jatropha curcas* kernel cake: effect on performance characteristics of goat. *Int. Res. J. Biotech.*, **1**(2): 19-22.

BELEWU M.A., ENIOLORUNDA O.O. & ILORI G.I., 2010a. Response of goat to fungi (*Rhizopus oligosporus*, *Rhizopus nigrican*) treated *Jatropha curcas* kernel cake. *Arch. Appl. Sci. Res.*, **2**(4): 255-261.

BELEWU M.A., BELEWU K.Y. & OGUNSOLA F.O., 2010b. Nutritive value of dietary fungi treated *Jatropha curcas* kernel cake: voluntary intake, growth and digestibility coefficient of goat. *Agric. Biol. J. N. Am.*, **1**(2): 135-138.

BELEWU M.A. & SAM R., 2010d. Solid state fermentation of *Jatropha curcas* kernel cake: proximate composition and antinutritional components. *J. Yeast. Fungal. Res.*, **1**(3): 44-46.

BELEWU M.A., AHMED O. & IBRAHIM S.O., 2011a. Solid state fermentation of *Jatropha curcas* kernel cake with cocktail fungi. *Int. J. Biosci.*, **1**(1): 12-19.

BELEWU M.A., BELEWU K.Y. & LAWAL I.A., 2011b. Cocktail of fungi blend on *Jatropha curcas* cake: effect on feed intake and blood parameters of goats. *Libyan Agric. Res. Cen. J. Intl.*, **2**(3): 138-143.

BELEWU M.A., BELEWU K.Y. & POPOOLA L.A., 2011c. Effect of cocktail of fungi blend on the digestibility coefficient and digestible nutrients of goat (*Capra hircus*). *Br. Biotechnol. J.*, **1**(2): 46-52.

BLUMBERG P.M. et al., 1987. Phorbol esters as probes of the modulatory site on protein kinase C – an overview. *Bot. J. Linean Soc.*, **94**(1-2): 283-292.

BRAND D., PANDEY A., ROUSSOS S. & SOCCOL C.R., 2000. Biological detoxification of coffee husk by filamentous fungi using a solid state fermentation system. *Enzyme Microbial Technol.*, **27**(1-2): 127-133.

BRUNETON J., 2008. Pharmacognosy, Phytochemistry, Medicinal plants (2nd edition). Lavoisier Publishing, Paris, 1136p.

CHERIAN M. & RACKIS J.J., 1980. Phytic acid interactions in food systems. *Crit. Rev. Food Sci. Nutr.*, **13**(4): 297-335.

CHIVANDI E., MTIMUNI J.P., READ J.S. & MAKUZA S.M., 2004. Effects of processing method on phorbol esters concentration, total phenolics, trypsin inhibitor activity and the proximate

composition of the Zimbabwean *Jatropha curcas* provenance: a potential livestock feed. *Pak. J. Biol. Sci*., **7**(6): 1001-1005.

De BARROS C.R.M. et al., 2011. The potential of white-rot fungi to degrade phorbol esters of *Jatropha curcas* L. seed cake. *Eng. Life Sci.* **11**(1): 107-110.

DEVAPPA R.K. et al., 2010a. Quality of biodiesel prepared from phorbol ester extracted *Jatropha curcas* oil. *J. Am. Oil Chem. Soc*., **87**(6): 697-704.

DEVAPPA R.K., MAKKAR H.P.S. & BECKER K., 2010b. Jatropha diterpenes: a review. *J. Am. Oil Chem. Soc.*, **88** (3): 301-322.

DINIS. M.J. et al., 2009. Modification of wheat straw lignin by solid state fermentation with white-rot fungi. *Bioresource Technol.*, **100**(20): 4829-4835.

DOS SANTOS M.M. et al., 2004. Thermal denaturation: is solid-state fermentation really a good technology for the production of enzymes? *Bioresource Technol.*, **93**(3): 261-268.

ELYAS S.H.A., EL TINAY A.H., YOUSIF N.E. & ELSHEIKH E.A.E., 2002. Effect of natural fermentation on nutritive value and *in vitro* protein digestibility of pearl millet. *Food Chem.*, **78**(1): 75-79.

ERDMAN J.W., 1979. Oilseed phytates: nutritional implications. *J. Am. Oil Chem. Soc*., **56**(8): 736-741.

EVANS F.J. & EDWARDS M.C., 1987. Activity correlations in the phorbol esters series. *Bot. J. Linean Soc*., **94**(1-2): 231-246.

FAOZIYAT S.A. et al., 2014. Aspergillus-fermented Jatropha curcas seed cake: proximate composition and effects on biochemical indices in Wistar rats. *Biol. Lett*., **41**(1): 37-46.

GANDHI V.M., CHERIAN K.M. & MULKY M.J., 1995. Toxicological studies on ratanjyot oil. *Food Chem. Toxicol*., **33**(1): 39-42.

GARRET R.H. & GRISHAM C.M., 2000. Biochimie. Bruxelles : De Boeck Université.

GAUR S., 2009. Development and evaluation of an effective process for the recovery of oil and detoxification of meal from *Jatropha curcas*. Master of Science in Chemical Engineering: Missouri University of Science and Technology (USA).

GINWAL H.S., RAWAT P.S. & SRIVASTAVA R.L., 2004. Seed source variation in growth performance and oil yield of *Jatropha curcas* L. in Central India. *Silvae Genetica*, **53**(4): 186-192.

GIRBÉS T. et al., 2004. Description, distribution, activity and phylogenetic relationship of ribosome-inactivating proteins in plants, fungi and bacteria. *Mini Rev. Med. Chem.*, **4**(5): 461-476.

GODOY M.G. et al., 2009. Use of a low-cost methodology for biodetoxification of castor bean waste and lipase production. *Enzyme Microbial Technol.*, **44**(5): 317-322.

GOEL G., MAKKAR H.P.S., FRANCIS G. & BECKER K., 2007. Phorbol esters: structure, biological activity, and toxicity in animals. *Int. J. Toxicol.*, **26**(4): 279-288.

GORDON J.A. & MARQUARDT M.D., 1974. Factors affecting hemagglutination by concavalin A and soybean agglutinin. *BBA, Biomembr.*, **332**(2): 136-144.

GRAMINHA E.B.N. et al., 2008. Enzyme production by solid-state fermentation: application to animal nutrition. *Anim. Feed Sci. Technol.*, **144**(1-2): 1-22.

HAAS W. & MITTELBACH M., 2000. Detoxification experiments with the seed oil from *Jatropha curcas* L. *Ind. Crops Prod.*, **12**(2): 111-118.

HAAS W., STERK H. & MITTELBACH M., 2002. Novel 12-deoxy-16-hydroxyphorbol diesters isolated from the seed oil of *Jatropha curcas*. *J. Nat. Prod.*, **65**(10): 1434-1440.

HECKER E., 1987. Tumour promoters of the irritant diterpene ester type as risk factors of cancer in man. *Bot. J. Linean Soc.*, **94**(1-2): 197-219.

HIROTA M. et al., 1988. A new tumor promoter from seed oil of *Jatropha curcas* L., an intramolecular diester of 12-deoxy-16-hydroxyphorbol. *Cancer Res.*,**48**: 5800-5804.

HOPKINS W.G., 2003. *Physiologie végétale*. Bruxelles : De Boeck Université.

HOSTETTMANN K. & MARSTON A., 2005. Saponins. Chemistry and pharmacology of natural products. Cambridge University Press, 549p.

JOHNSON W., 2003. Invitation à la chimie organique. Bruxelles : De Boeck Université.

JONGBLOED A.W., MROZ Z. & KEMME P.A., 1992. The effect of supplementary *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total

phosphorus, and phytic acid in different sections of the alimentary tract. *J. Anim. Sci*., **70**(4): 1159-1168.

JOSHI C., MATHUR P. & KHARE S.K., 2011. Degradation of phorbol esters by *Pseudomonas aeruginosa* PseA during solid-state fermentation of deoiled *Jatropha curcas* seed cake. *Bioresource Technol.*, **102**(7): 4815-4819.

KADNER S.S., KATZ J., LEVITZ M. & FINLAY T.H., 1985. The 65-kDa phorbol-diester hydrolase in mouse plasma is esterase 1 and is immunologically distinct from the 56-kDa phorbol-diester hydrolase in mouse liver. *J. Biol. Chem.*, **260**(29): 15604-15609.

KAUFMANN P.B. et al., 1999. Natural products from plants. Boca Raton, FL, USA: CRC Press LLC.

KAUL R., READ J. & MATTIASSON B., 1991. Screening for plants lectins by latex agglutination tests. *Phytochemistry*, **30**(12): 4005-4009.

KIKKAWA U., TAKAI Y., TANAKA Y., MIYAKE R. & NISHIZUKA Y., 1983. Protein kinase C as a possible receptor protein of tumor-promoting phorbol esters. *J. Biol. Chem.*, **258**(19): 11442-11445.

KUMAR V., SINHA A.K., MAKKAR H.P.S., De BOECK G. & BECKER K., 2011. Phytate and phytase in fish nutrition. *J. Anim. Physiol. Anim. Nutr*., **96**(3): 335-364.

LAJOLO F.M. & GENOVESE M.I., 2002. Nutritional significance of lectins and enzyme inhibitors from legumes. *J. Agricult. Food Chem.*, **50** (22): 6592-6598.

LI C.-Y. et al., 2010. Toxicity of *Jatropha curcas* phorbol esters in mice. *Food Chem. Toxicol*., **48**: 620-626.

LIENER I.E., 1979. The nutritional significance of plant protease inhibitors. *Proc. Nutr. Soc*., **38**(1): 109-113.

LIN J. et al., 2003. Cloning and expression of curcin, a ribosome-inactivating protein from the seeds of *Jatropha curcas*. *Acta Bot. Sin*., **45**(7): 858-863.

LIN J. et al., 2010. Purification and characterization of curcin, a toxic lectin from the seed of *Jatropha curcas*. *Prep. Biochem. Biotechnol*., **40**(2): 107-118.

LONGO M.A., DEIVE F.J., DOMINGUEZ A. & SANROMAN M., 2008. Solid-state fermentation for food and feed application (379-411). *In*: Current developments in solid-state fermentation. Pandey A., Soccol C.R., Larroche C., eds.- New Dehli : Asiatech Publishers, Inc.- 517p.

LUO M.J. et al., 2007. Cloning, expression, and antitumor activity of recombinant protein of curcin. *Russ. J. Plant Physiol.*, **54**(2): 202-206.

MADEIRA J.V., MACEDO J.A. & MACEDO G.A., 2011. Detoxification of castor residues and the simultaneous production of tannase and phytase by solid-state fermentation using *Paecilomyces variotii*. *Bioresource Technol.*, **102**(15): 7343-7348.

MAKKAR H.P.S., ADERIBIGBE A.O. & BECKER K., 1998a. Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. *Food Chem.*, **62**(2): 207-215.

MAKKAR H.P.S. & BECKER K., 1999. Nutritional studies on rats and fish (carp *Cyprinus carpio*) fed diets containing unheated and heated *Jatropha curcas* meal of a non-toxic provenance. *Plant Foods Hum. Nutr.*, **53**(3): 183-192.

MAKKAR H.P.S. & BECKER K., 2009a. *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. *Eur. J. Lipid Sci. Technol.*, **111**(8): 773-787.

MAKKAR H.P.S, BECKER K. & SCHMOOK B., 1998b. Edible provenances of *Jatropha curcas* from Quintana Roo state of Mexico and effect of roasting on antinutrient and toxic factors in seeds. *Plant Food Hum. Nutr.*, **52**(1), 31-36.

MAKKAR H.P.S., BECKER K., SPORER F. & WINK M., 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. *J. Agric. Food Chem.*, **45**: 3152-3157.

MAKKAR H.P.S., MAES J., De GREYT W. & BECKER K., 2009b. Removal and degradation of phorbol ester during pre-treatment and transesterification of *Jatropha curcas* oil. *J. Am. Oil Chem. Soc.*, **86**(2): 173-181.

MARSHALL G.T., KLOCKE J.A., LIN L.-J. & KINGHORN A.D., 1985. Effects of diterpene esters of tigliane, daphnane, and lathyrane types on pink bollworm, *Pectinophora gossipiella* saunders (Lepidoptera: gelechiidae). *J. Chem. Ecol.*, **11**(2): 191-206.

MARTINEZ-HERRERA J. et al., 2006. Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. *Food Chem*., **96**(1): 80-89.

MENTLEIN R., 1986. The tumor promoter 12-O-tetradecanoyl phorbol 13-acetate and regulatory diacylglycerols are substrate for the same carboxylesterase. *J. Biol. Chem.*, **261**(17): 7816-7818.

MILLARD C.B. & Leclaire R.D., 2008. Ricin and related toxins: review and perspective. *In:* Romano J.A., Lukey B.J. & Salem H., eds. Chemical warfare agents: chemistry, pharmacology, toxicology, and therapeutics. 2nd ed. Boca Raton, FL, USA: CRC Press LLC, 423-467.

OSENI O.A. & AKINDAHUNSI A.A., 2011. Some Phytochemical properties and effect of fermentation on the seed of *Jatropha curcas* L. *Am. J. Food Technol.*, **6**(2): 158-165.

PANDEY A., 1992. Recent process developments in solid-state fermentation. *Process Biochem.*, **27**(2): 109-117.

PANDEY A., 2003. Solid-state fermentation. *Biochem. Eng. J.*, **13**(2-3): 81-84.

PANDEY A.; SOCCOL C.R. et MITCHELL D., 2000. New developments in solid state fermentation: I-bioprocesses and products. *Process Biochem.*, **35**(10): 1153-1169.

PERTINO M. et al., 2007. Biotransformation of jatrophone by *Aspergillus niger* ATCC 16404. Zeitschrift Für Naturforschung B – *J. Chem. Sci*., **62**: 275-279.

PORRES J.M., LÓPEZ-JURADO M., ARANDA P. & URBANO G., 2004: Bioavailability of phytic acid-phosphorus and magnesium from lentils (*Lens culinaris* m.) in growing rats: influence of thermal treatment and vitamin-mineral supplementation. *Nutrition*, **20**(9): 794-799.

QIN W., 2005. Expression of a ribosome inactivating protein (curcin 2) in *Jatropha curcas* is induced by stress. *J. Biosci*., **30**(3): 351-357.

RAIMBAULT M., 1998. General and microbiological aspects of solid substrate fermentation. *Electr. J. Biotechnol.*, **1**(3): 174-188.

RACKIS J.J. & GUMBMANN M.R., 1981. Protease inhibitors: physiological properties and nutritional significance. *In:* Ory R.L. Antinutrients and natural toxicants in foods. Westport, CT, USA: Food and Nutrition Press.

RAKSHIT K.D. et al., 2008. Toxicity studies of detoxified jatropha meal (*Jatropha curcas*) in rats. *Food Chem. Toxicol.*, **46**(12): 3621-3625.

RAVEN P.H., EVERT R.F. & EICHHORN S.E., 2003. Biologie végétale. Bruxelles : De Boeck Université.

REDDY N.R. & PIERSON M.D., 1994. Reduction in antinutritional and toxic components in plants foods by fermentation. *Food Res. Int.*, **27**(3), 281-290.

ROSA T.D.S., CASTRO A.M., TORRES A.G. & FREIRE D.M.G., 2010. Analysis of nutritional composition and detoxification of *Jatropha curcas* cake after solid-state fermentation (12-29). *In*: 32nd Symposium on Biotechnology for Fuels and Chemicals, 19-22 April 2010, Clearwater Beach, Florida.

RYAN C.A., 1990. Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annu. Rev. Phytopathol.*, **28**: 425-449.

SCHUSTER E., DUNN-COLEMAN N., FRISVAD J.C. & VAN DIJCK P.W.M., 2002. On the safety of *Aspergillus niger* – a review. *Appl. Microbiol. Biotechnol.*, **59**(4-5): 426-435.

SEIGLER D.S., 1998. Plant secondary metabolism. Norwell, MA, USA: Kluwer Academic Publishers.

SHOYAB M.; WARREN T.C. et TODARO G.J, 1981. Isolation and characterization of an ester hydrolase active on phorbol diesters from murine liver. *J. Biol. Chem.*, **256**(23): 12539-12534.

SILINSKY E.M. & SEARL T., 2003. Phorbol esters and neurotransmitter release: more than just protein kinase C? *Br. J. Pharmacol.*, **138**(7): 1191-1201.

SIMONS P.C. et al., 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br. J. Nutr.*, **64**(2): 525-540.

SPARG S.G., LIGHT M.E. et Van STADEN J., 2004. Biological activities and distribution of plant saponins. Journalof Ethnopharmacology, **94**(2-3): 219-243.

SPILLER G. & SPILLER M., 2005. What's with fiber. Basic health publications, 224p.

STAUBMANN R. et al., 1997. Biogas production from *Jatropha curcas* press-cake. *Appl. Biochem. Biotechnol.*, **63-65**(1): 457-467.

STIRPE F. & BARBIERI L., 1986. Ribosome-inactivating proteins up to date. *FEBS Lett*., **195**(1-2): 1-8.

STIRPE F. & BATTELLI M.G., 2006. Ribosome-inactivating proteins: progress and problems. *Cell. Mol. Life Sci*., **63**(16): 1850-1866.

VOLLHART K.P.C. & SCHORE N.E., 2004. Traité de chimie organique. Bruxelles : De Boeck Université.

WALKER R., 2002. Risk assessment of ochratoxin: current views of the European scientific committee on food, the JECFA and the CODEX committee on food additives and contaminants (249-256). *In*: Mycotoxins and food safety – Advances in experimental medicine and biology, volume 504. DeVries J.W., Trucksess M.W. & Jackson L.S. eds.- New-York: Kluwer Academic/Plenum Publishers.

WINA E., TANGENDJAJA B., PASARIBU T. & PURWADARIA T., 2010. Broiler performance fed *Jatropha curcas* seed meal detoxified by fermentation, physic and chemical treatments. *Indon. J. Anim. Vet. Sci.*, **15**(3): 174-181.

XIAO J. ; ZHANG H.; NIU L.; WANG X. et LU X., 2011. Evaluation of detoxification methods on toxic and antinutritional composition and nutritional quality of proteins in *Jatropha curcas* meal. *J. Agricult. Food Chem.*, **59**(8): 4040-4044.

## CHAPITRE II

**Incorporation de l'amande de *Jatropha curcas* déshuilée dans la ration de poulets de chair**

**Digestibility of solvent-treated *Jatropha curcas* kernel by broiler chickens in Senegal**

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**Abstract**

*Jatropha curcas* is a drought-resistant shrub belonging to the *Euphorbiaceae* family. The kernel contains approximately 60% lipid in dry matter and the meal obtained after oil extraction could be an exceptional source of protein for family poultry farming, in the absence of curcin and, especially, some diterpene derivatives phorbol esters that are partially lipophilic. The nutrient digestibility of *J. curcas* Kernel Meal (JKM), obtained after partial physico chemical de-oiling was thus evaluated in broilers chickens.

Twenty broiler chickens, 6 weeks old, were maintained in individual metabolic cages and divided into 4 groups of 5 animals, according to a 4x4 Latin square design where de-oiled JKM was incorporated into grinded corn at 0, 4, 8 and 12% levels (diets 0J, 4J, 8J and 12J), allowing measurement of nutrient digestibility by the differential method. The Dry Matter (DM) and Organic Matter (OM) digestibility of diets was affected to allow extent by JKM (85 and 86% in 0J and 81% in 12J, respectively) in such a way that DM and OM digestibility of JKM was estimated to be close to 50%. The Ether Extract (EE) digestibility of JKM remained high, at about 90%, while Crude Protein (CP) and Crude Fiber (CF) digestibility were largely impacted by JKM, with values closed to 40% at the highest levels of incorporation. *J. curcas* kernel presents various nutrient digestibilities but has adverse effects on CP and CF digestibility of the diet. The effects of an additional heat treatment on JKM remain to be assessed.

**Keywords:** broiler chickens, *Jatropha curcas*, digestibility

## 1. Introduction

In tropical environment, nutrient and feed supplies are limiting factor in broiler production due to competition between man and poultry, and also to poorly locally available or valuable feed resources (Steinfeld *et al*., 2006). Increasing need for high quality protein in poultry livestock stresses the search

for new sources of protein that do not interfere with the food security rights. Thus the non-food oil seeds and their by-products could be feed of choice provided that they are free of toxic and anti-nutritional factors (Sivaramakrishnan & Gangadharan, 2009).

*Jatropha curcas is* a wild drought-resistant shrub belonging to the *Euphorbiaceae* family, which can grow in marginal wastelands and is often used for soil erosion control (Levingston & Zamora, 1983). It is easily propagated by cutting (Heller, 1996) and planted as a fence to protect fields or concessions because it is not consumed by animals. Up to now, the plant is has not yet been really domesticated but all parts of the plant are used for traditional and veterinary purposes (Duke, 2002). The nut of *J. curcas* is commonly known as physic or purging nut, but also is called *pourghere* in french, or *tabanani* in Senegal. The fruit, main crop material, contain 1-4 seeds, consisting of shells (35%) and kernel (65%). The oil content of the seeds, is about 22 to 48% (Becker & Makkar, 2008), traditionally obtained by mechanical pressure (Beerens, 2007) or extracted with solvents. It can be converted into bio-diesel by transesterification (Foidl *et al*., 1996) or used in traditional oil lamps and for soaps production (Henning, 2003). About one fifth of the fatty acids are saturated (Vaknin *et al*., 2011). Jatropha cake is nitrogen-rich and a very good soil fertilizer (Heller, 1996).

The kernel meal, obtained after oil extraction, contains about 500g/kg of crude protein in the dry matter (Aderibigbe et al., 1997) and has a level of essential amino acids, except lysine, comparable to the reference values recommended by FAO (Makkar et al., 1998).

The use of jatropha meal in animal feed remains however limited. The seed was found to be toxic for children (Abdu-Aguye & Sannusi, 1986) and for several species such as cattle (Ahmed & Adam, 1979a), sheep and goat (Ahmed & Adam, 1979b), rat and rabbit (Gandhi *et al*., 1995) and fish (Becker & Makkar, 1998).

Jatropha toxicity was initially suggested to be due to curcin, a lectin with a sharp inhibitory effect on protein synthesis (Stirpe *et al*., 1976). It was then established that the main toxic compounds in kernel, oil and cake, are diterpene derivatives classified as phorbol esters (Makkar *et al*., 1997). They act on biological membranes and directly activate protein kinase C, an enzyme which plays an essential role in the transduction signal regulating cell growth and differentiation (Aitken, 1987). In addition, the nut

by-products contain anti-nutritional factors such as trypsin inhibitors, saponins and phytate which interfere with digestive process in animals (Aderibigbe *et al.*, 1997).

Seeds, cake or oil should be detoxified before being used as feed. Detoxification methods are essentially chemical. Martinez-Herrera *et al.* (2006) decreased the phorbol esters content in oil cakes by 98%, using an ethanol extraction. The results were better than using methanol (Aregheore *et al.*, 2003; Ahmed & Salimon, 2009; Gaur, 2009; Devappa *et al.*, 2010) or hexane (Chivandi *et al.*, 2004; Rakshit *et al.*, 2008) for which authors reached out extraction rates of 92 and 89% respectively. Biological methods of detoxication were described. Belewu *et al.* (2010) reduced phorbol esters by 77% and other anti-nutritional substances such as saponins by 95% by inoculating jatropha cake with *Aspergillus niger*.

The objective of this study was to investigate in Senegal, the nutrient digestibility in broiler chickens of *J. curcas* kernel meal physic-chemically de-oiled in order to remove phorbol esters out of the product.

## 2. Materials and methods

### 2.1. Location of the experiment

The experiment was conducted in *Ecole Nationale Supérieure d'Agriculture* (*ENSA*), University of Thies (Senegal), in 2012 and was repeated in 2013 at the beginning of the rainy season (June – July). This period was characterized by an average temperature ranging from 25.9 to 35.4°C and a relative humidity from 40.4 to 80.5%.

### 2.2. Preparation of the jatropha kernel meal and diet formulation

Thirty five kg of mature and dry seeds of *J. curcas* were collected each year from Dialacoto, Senegal. The seeds were cracked and unshelled manually to obtain kernels, which were grinded to get a Jatropha Kernel Paste (JKP).

A residual level of Ether Extract (EE) lower than 100g/kg Dry Matter (DM) was judged to be adequate to perform the trial. Oil extraction with petroleum ether (boiling range 40-60°C) was assessed diluting 1vol ether in 1vol JKP, assuming a homogeneous distribution of the solvent in the mass. The residual oil content has peaked at 400g/kg DM. Consequently, it was decided to combine a mechanical pressure with solvent extraction. Finally, the JKP was defatted by 4 alternated phases of 24 h soaking

in a bucket of petroleum ether (1:1vol) and pressing with a manual perforated cylinder press. After last soaking, the paste was spread left to dry for 24 h in order to remove the residual ether.

Defatted Jatropha Kernel Meal (JKM) looked like a fine white powder. It was then incorporated into grinded corn at levels of 40, 80 and 120g/kg (diets 4J, 8J and 12J). The control diet (0J) consists of grinded corn.

### 2.3. Animals and housing

Two consecutive years, 20 unsexed broiler chickens, strain Ross 308, 6 weeks old, initial weight 1848±314g in 2012 and 1411±160g in 2013, were used. Animals were divided into 4 groups of 5 subjects, corresponding to the four dietary treatments and were maintained in individual metabolic cages according to the model described by Dahouda *et al.* (2009). The experimental design was a randomized complete block with five repetitions corresponding to the arrangement in metabolism cages in blocks of five boxes arranged in a ventilated room, placed at 50cm above the ground. The blocks that have made from galvanized sheet were each divided into five boxes (l x w x h: 60 x 30 x 40cm each). The boxes were screened in their upper and front portions, but were sealed on their side and rear parts. The front face equipped with a feeder and a drinker, also galvanized sheet. They opened in their upper part while the lower part was equipped with a tray that allowed collecting separately feaces as well as water and feed spills.

At the beginning of the experiments, animals were adapted to their new environment for one week during which they received the control diet. This period was planned to allow animals to accommodate to their environment and to estimate feed intake. Thereafter, each group received for 7 days a given ration which was then randomly re-allocated to another group the three following weeks, according to a Latin square design. Each week, measurements were performed from day3 to day7.

Feed was weighed early in the morning and provided 2 times per day. For each animal, food refusals were collected and weighed the day following the distribution. During the test, water was available *ad libitum*.

### 2.4. Fattening performance and Nutrients digestibility

During the experiment, the weight of the broiler chickens was determined at the beginning and the end of each Latin square period. The daily individual amounts of feed intake (distributed minus refused)

and feaces excreted were recorded and sub-sampled for dry matter determination. At the end of the experiment, materials collected were crushed and pooled by groups and by period for chemical analysis. Apparent Digestibility Coefficients (ADC) were thus determined on each animal for dry matter and on groups-period for nutrients, following the formula:

ADC (%) = [(nutrients intake – nutrients in excretas)/nutrients intake] x 100.

The Differential Apparent Digestibility Coefficient (Diff ADC) for the jatropha kernel meal incorporated at X% (Diff ADC at X) was also determined, compared to that of corn, on each animal for dry matter and on groups-periods for nutrients, following the formula:

Diff ADC at X (%) = [ADC nutrient at X% – (ADC corn nutrient x (1-X))] / (1-X)

### 2.5. Chemical analyses

Chemical analyses were performed according to the procedures of AOAC (1990). Crude Protein (CP) was determined by the Kjeldahl method (N x 6.25), Ether Extract (EE) by the Soxhlet method and Crude Fiber (CF) by the method of Weende.

The following values were calculated from those measured:

Organic Matter (OM) = 100 – Ash

Non-Nitrogen Extract (NNE) = OM – EE – CP – CF

### 2.6. Statistical analysis

Data were analyzed according to the following general linear model (proc GLM, SAS…):

$Yijkl = \mu + Ti + Pj + Uk + Sl + Am(Sl) + Eijklm$

Where

Yijkl = the experimental data

Ti = fixed effect of the treatment i (i = 1 to 4)

Pj = fixed effect of the period j (j = 1 to 4)

Uk = random effect of the experimental group k (k = 1 to 4)

Sl = random effect of the square (or year) l (l = 1 to 2)

Am(S1) = random effect of animal m (m = 1 to 5) nested within square l

Eijklm = random residual effect, N[0, 1]

Animal effect is optional according to the level of the experiment unit.

**Multiple comparisons were performed according to Student's test, adjusted by Tukey method.**

## 3. Results

### 3.1. Chemical composition of diets

**Table 1** showed chemical composition of the raw and physico-chemically de-oiled kernels and of the different experimental diets.

The proximate compositions of refusals and feaces are indicated in **Table 2**. Kernel contained more than 600g/kg in Dry Matter (DM) of Ether Extract (EE) and about a quarter of Crude Protein (CP), while Crude Fiber (CF), Ash and Non-Nitrogen Extract (NNE) remained close to 50g/kg DM. The level of EE in kernel meal was quite lower than the objective of 100g/kg DM. As a consequence, CP represented more than a half of material, followed with NNE (about a quarter of DM). While CF remained close to 50g/kg DM, Ash increased to about 100g/kg DM. As a consequence, the levels of CP in diets increased with kernel incorporation (from about 100 to 130g/kg DM), by contrast to CF and EE, the value of which being close to 30 and 60g/kg DM respectively.

Overall, refusals were characterized by similar values as to feeds. Faeces contained about twice the value of CP observed in feeds, i.e., increase with the level of kernel incorporation, and about the triple of CF. In parallel, the NNE remained close to 600g/kg DM.

**Table 1:** Proximate composition of raw materials

| | DM | Chemical composition (% in DM) | | | | | |
|---|---|---|---|---|---|---|---|
| | (%) | OM | CP | EE | CF | Ash | NNE |
| **Kernel** | 95.7 | 95.8 | 24.6 | 60.6 | 6.4 | 4.2 | 4.2 |
| **Kernel meal** | 90.9 | 89.4 | 51.8 | 6.5 | 4.7 | 10.6 | 26.4 |
| **Diet 0J** | 89.4 | 98.1 | 9.0 | 5.0 | 3.0 | 1.9 | 81.2 |
| **Diet 4J** | 89.7 | 97.8 | 10.4 | 5.4 | 3.0 | 2.3 | 79.0 |
| **Diet 8J** | 89.7 | 97.3 | 11.4 | 5.0 | 2.6 | 2.7 | 78.4 |
| **Diet 12J** | 89.8 | 96.9 | 12.9 | 6.3 | 3.3 | 3.1 | 74.4 |

DM: Dry Matter, MO: Organic Matter, CP: Crude Protein, EE: Ether Extract, CF: Crude Fiber, NNE: Non-Nitrogen Extract, diet 0J: control diet (grinded corn), diet 4J to 12J: 4 to 12% jatropha kernel meal in grinded corn.

**Table 2:** Composition of refusal and faeces of broiler chickens offered different incorporation level of *Jatropha curcas* meal in diet

| | DM | Chemical composition (% in DM) | | | | | |
|---|---|---|---|---|---|---|---|
| | (%) | OM | CP | EE | CF | Ash | NNE |
| **Refusals 0J** | 90.3 | 96.7 | 9.5 | 3.2 | 2.7 | 3.3 | 79.0 |
| **Refusals 4J** | 91.5 | 97.0 | 11.5 | 3.5 | 2.6 | 3.0 | 78.1 |
| **Refusals 8J** | 91.8 | 96.8 | 13.3 | 3.7 | 2.8 | 3.2 | 77.3 |
| **Refusals 12J** | 90.8 | 96.8 | 14.3 | 4.4 | 2.7 | 3.2 | 77.1 |
| **Faeces 0J** | 92.8 | 94.0 | 19.4 | 5.0 | 7.7 | 6.0 | 63.7 |
| **Faeces 4J** | 92.9 | 93.2 | 21.9 | 5.0 | 7.7 | 6.8 | 58.7 |
| **Faeces 8J** | 92.9 | 92.1 | 22.6 | 5.0 | 7.1 | 7.9 | 60.3 |
| **Faeces 12J** | 92.9 | 92.1 | 23.6 | 6.3 | 7.9 | 7.9 | 54.3 |

DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, EE: Ether Extract, CF: Crude Fiber, NNE: Non-Nitrogen Extract, diet 0J: control diet (grinded corn), diet 4J to 12J: 4 to 12% jatropha kernel meal in grinded corn.

### 3.2. Fattening performance and digestibility

**Table 3** presents animal performance of broiler chickens fed graded levels of processed jatropha kernel meal in grinded corn. Neither mortality nor signs of toxicity were recorded. Control animals had a higher level of daily intake at about 50g/d than other groups. Feed intake decreased linearly with the level of jatropha incorporation: about 2g per unit increase. Value in the 12J group reached about half that of the control group. Fecal DM excretion also linearly decreased with jatropha incorporation, at about 1g at each step of increase. The treatments had dramatic influences on weight gains. If a quasi-steady state was observed in the control group, animals from 4J lost about 9g/d, values doubled at levels 8J and 12J.

Owing to a proportionally low level of DM fecal excretion when compared to feed intake, Apparent Dry Matter Digestibility (ADMD) coefficient was higher than 80% in the different group (**Table 4**) but decreased significantly ($P<0.05$) and linearly with the level of incorporation of *J. curcas*, allowing to extrapolate a ADMD of 53.6% at a theoretical 100% incorporation of *J. curcas* meal.

The Apparent Organic Matter Digestibility (AOMD) followed a similar evolution as to DM, with close but slightly higher values. The Apparent Crude Protein Digestibility (ACPD), as for it, differed between groups and decreased sharply with the level of *J. curcas* incorporation, with 34% at the 8J level of incorporation. The Apparent Ether Extract Digestibility (AEED) was high at about 84% and

did not showed significant difference between groups (P>0.05) but without clear evolution, the 4J diet showing the highest values at 87%. The Apparent Crude Fiber Digestibility (ACFD) was the lowest out of all other components and showed similar evolution as to EE: the difference between groups was significant but values decreased with the levels of *J. curcas* incorporation, reaching 0 at the highest level.

The differential apparent digestibility coefficient (**table 5**) of the jatropha kernel meal was obtained compared to the control diet which is the corn. Thus, values ranging from 52.70% for 4J to 63.99% for 12J were obtained from the dry matter. The same trend was observed with the organic matter with the highest value at 8J. As against, as regards the crude protein, the differential apparent digestibility remains negative whatever supplemented diet.

**Table 3:** Effect of different incorporation level of *Jatropha curcas* meal deoiled for 24 hours (4 times) in diet of broiler chickens on dry matter feed intake and feaces production, and on weight gain.

| | **Treatments** | | | | **P>F** | **SEM** |
|---|---|---|---|---|---|---|
| | **Control** | **4J** | **8J** | **12J** | | |
| **DM Feed intake (g/day)** | 49.3±2.9$^{a}$ | 40.0±4.2$^{b}$ | 31.8±4.6$^{c}$ | 26.4±5.9$^{c}$ | 0.000 | 1.20 |
| **DM Feaces (g/day)** | 7.5±0.6$^{a}$ | 6.4±0.1$^{ab}$ | 5.1±0.4$^{bc}$ | 4.3±0.3$^{c}$ | 0.005 | 0.39 |
| **Weight gain (g)** | 4.0±2.4$^{a}$ | -36.8±10.9$^{ab}$ | -72.0±56.2$^{b}$ | -74.8±13.8$^{b}$ | 0.002 | 8.50 |

SEM = Standard Error of the Mean.

$^{a, b, c}$ = means with different superscripts on the same row differ significantly (P<0.05).

**Table 4:** Effect of different level of incorporation of *Jatropha curcas* meal deoiled for 24 hours (4 times) in diet of broiler chickens on apparent nutrient digestibility.

| | **Treatments** | | | | **P>F** | **SEM** |
|---|---|---|---|---|---|---|
| | **Control** | **4J** | **8J** | **12J** | | |
| **ADMD (%)** | 84.9$^{a}$ | 83.0$^{a}$ | 82.6$^{a}$ | 80.8$^{b}$ | 0.030 | 0.38 |
| **AOMD (%)** | 86.2$^{a}$ | 84.0$^{b}$ | 85.1$^{ab}$ | 81.4$^{c}$ | 0.001 | 0.38 |
| **AEED (%)** | 83.5 | 87.4 | 77.9 | 85.9 | 0.618 | 5.29 |
| **ACPD (%)** | 51.7$^{a}$ | 41.3$^{ab}$ | 34.4$^{b}$ | 39.4$^{ab}$ | 0.019 | 2.74 |
| **ACFD (%)** | 37.6 | 29.2 | 0.5 | 38.2 | 0.166 | 11.45 |

ADMD: Apparent Dry Matter Digestibility, AOMD: Apparent Organic Matter Digestibility, AEED: Apparent Ether Extract Digestibility, ACPD: Apparent Crude Protein Digestibility, ACFD: Apparent Crude Fiber Digestibility. Different superscripts within one column indicate differences between groups (P<0.05).

**Table 5:** Differential apparent digestibility coefficient of *Jatropha curcas* meal deoiled for 24 hours (4 times) based on its incorporation in diets.

| | Treatments | | | P>F | SEM |
|---|---|---|---|---|---|
| | **4J** | **8J** | **12J** | | |
| **Diff ADMD (%)** | 52.7 | 61.7 | 64.0 | 0.010 | 9.73 |
| **Diff AOMD (%)** | 51.1 | 68.8 | 68.1 | 0.000 | 5.46 |
| **Diff ACPD (%)** | -70.1 | -85.2 | -0.4 | 0.800 | 77.68 |

Diff ADMD: Differential Apparent Dry Matter Digestibility, Diff AOMD: Differential Apparent Organic Matter Digestibility, Diff ACPD: Differential Apparent Crude Protein Digestibility.

## 4. Discussion

The extract of vegetable oil from seeds is mainly based on two processes which are mechanical pressing and solvent extraction. Mechanical screw press is a mean of oilseed crushing to small and medium scale (Zheng *et al.*, 2003). In order to fully remove weakly digestible sheathes, and thus to study the specific effects of kernels on poultry, seeds were shelled manually. After shell removing, the kernels contained between approximately 600g/kg in DM of EE. These values are somewhat higher than those obtained by Aderibigbe *et al.* (1997), Makkar *et al.* (1998), Martinez-Herrera *et al.* (1998), Achten (2010) and Kumar *et al.* (2010).

The following use of screw press did not permit oil extraction, but grinded the jatropha kernel as a paste. This confirms the negative effect of shelling on oil yield during crushing (Bereens, 2007). To overcome this difficulty, petroleum ether was used to allow oil extraction from the paste. The solvent is a special gasoline G type, colorless liquid, of low viscosity and very good solvent of greases (Brondeau *et al.*, 1999). It allows direct extraction by exhaustion. However, its flammability, toxicity and price indexed to oil prices are major disadvantages of its use (Johnson, 2008).

The de-oiling process used, yielded a meal containing about 6.5% ether extract in dry matter. This value is lower than those obtained by Aderibigbe *et al.* (1997) with a partial de-oiling, but higher than those obtained by the same authors and Makkar *et al.* (1998) with a totally defatted meal. These differences in results compared to the method used, can be explained by the process of de-oiling. Indeed, the last authors implemented a soxhlet de-oiling which eliminates all the fat of the matter. Soaking method used presently, more compatible with a field experiment, left a significant amount of fat.

Chemical analyzes made on JKM showed that it mainly consist of crude protein and EE (580g/kg in DM). The levels of crude protein and ash were similar with those obtained by Aderibigbe *et al*. (1997), Makkar *et al*. (1997), Makkar *et al*. (1998), Achten *et al*. (2008) and Lago (2009), but the values in EE and especially in crude fiber were higher.

Over the two years, the crude protein obtained values of jatropha kernel meal remain higher than that the soybean meal (518 vs 457g/kg in DM) (Makkar et al., 1998), thus confirming the nutritional profile of de-oiled jatropha kernel. Diets offered for digestibility showed a crude protein content of between 8 and 13% DM, depending on the level of incorporation of the JKM. These values are far below those recommended for production (INRA, 1989).

In our study, the daily intake per broiler chicken was inversely dependent on the incorporation rate of the JKM resulting in lower weight gain. This decline is probably related of palatability as animals systematically reduced their consumption whenever they were exposed to jatropha. It was reported that feed intake is influenced by a variety of factors, such as taste, smell and texture of the diet (Temler *et al*., 1983). However, it must be noted that the experimenters did not detect any unpleasant smell or taste with regard to the kernel. It was also noted that the excretion of feaces was mechanically proportional to the ingestion of diets. Thus, animals that ingested the control diet showed, as expected, the highest values of feaces.

Dry matter intake and body weight gain were significantly ($P<0.05$) lower in all incorporated jatropha kernel groups in comparison with control group. This is probably due to the presence of phorbol esters and poor protein utilization in the diets (Aregheore *et al*., 2003). Phorbol esters which are the main toxins in *J. curcas* (Makkar & Becker, 2009) were found to be responsible for purgative and skin-irritant effects (Adolf *et al*., 1984).

The reduced body weight during experiments was due to both reduced intake but also poor protein utilization. In this respect, trypsin inhibitors and curcin are known to decrease the weight gain performance of animals (Francis *et al*., 2001) and were related to the level of jatropha kernel in the diet. Tryspin inhibitors are antinutritional factors which interfere with the physiological process of digestion in non-ruminants, leading to severe growth depression (White et al., 2000). Oladunjoye *et al.*

(2014) observed a similar growth depression due to residual anti-nutritional factors, by fermenting *Aspergillus niger* on jatropha kernel meal.

For defatted and untreated jatropha kernel meal, Aderibigbe *et al.* (1997) measured a trypsin inhibitor activity to about 20mg/g of sample. Heat treatments reduced this activity to 0.2mg/g of sample, showing the thermo labile character of the toxin. The effect of heat treatment was confirmed by Abou-Arab & Abou-Salem (2010). In our case, the kernel of jatropha was deffated without heat treatment. Trypsin inhibitors were still present and probably contribute to interference with the physiological digestive process in poultry. These observations are in agreement with those made by Makkar *et al.* (1998), Makkar & Becker (1999) and Kumar *et al.* (2010) who showed adverse physiological effects in monogastric and therefore a decrease in voluntary intake and reduced weight gain for animals subjected to diets with unheated jatropha kernel meal.

Furthermore, Makkar *et al.* (1997) showed that the most important toxic principle on jatropha seed is represented by phorbol esters which are thermo stable (Aregheore *et al.*, 2003). In addition, the mode of extraction of oil has an impact on the level of phorbol esters which remain high with a press extraction (Beerens, 2007). Chemical deoiling of jatropha kernel, followed by a physicochemical detoxification treatment does not cause a complete removal of toxic factors including phorbol esters (Kumar *et al.*, 2010). Its inclusion in diets thus caused a decrease in feed intake and a weight gain reduction on monogastrics. In spite of chemical treatments (sodium chloride and calcium hydroxide) that decreased phorbol esters and haemagglutination activity, Katole *et al.* (2011) also observed a reduced nutrient intake. Also, Annongu *et al.* (2010), confirmed by Abdel-Shafy & Nasr (2010), showed a tolerance in diets containing physico chemically treated jatropha kernel meal up to 15%. Beyond, mortality was recorded, showing a cumulative effect of toxic factors, including phorbol esters. Monogastrics show great sensibility to this compound in feed (Becker & Makkar, 1998). They showed intestinal irritation and thus feed rejection due to the residual effects of the toxins. In our study, despite the time jatropha kernel meal was offered, and in the two years of successive experiments, the animals did not show any signs of visible intoxication that could have an impact on their health. We only observed a decrease in voluntary ingestion of diets as soon as they contained jatropha, contrary to Sirisha *et al.* (2009) who observed in broiler chicken, clinical signs of

inappetance, depression, loss of body weight, lower feed intake, profuse greenish diarrhoea, pasty vent and restricted movement.

The apparent digestibility of dry matter, organic matter and ether extract remained over 80% for all diets. As against, concerning the apparent crude protein and crude fiber digestibility, values ranged from 60% to almost 0 by the rate of incorporation the JKM in the grinded corn. These results show a low nitrogen and fiber retention rate when compared of other nutrients.

Concerning the *in vitro* protein digestibility, lowest values were observed for defatted samples because of high content in trypsin inhibitor (Martinez-Herrera *et al*., 2006). Samples which were submitted to heat treatment, improved their digestibility by about 7% owing to the denaturation and inactivation of protease inhibitors (Carbonaro *et al*., 1997). These results were confirmed with carp by Kumar *et al.* (2010) whose obtained a good apparent digestibility of protein (89-92%) from the defatted jatropha kernel meal after phorbol esters removal and inactivation not only trypsin inhibitors but also lectin by heat treatment. Thus, the probable presence of trypsin inhibitors in the jatropha kernel meal used lowered apparent digestibility of proteins by interaction with proteolytic enzymes (Hajos *et al*., 1995). Using a enzymatic hydrolysis of phorbols, followed by washing with ethanol, Xiao *et al.* (2011) decreased by 100% phorbol esters and antinutritional components to tolerable levels. They increased also the *in vitro* protein digestibility by 11%. In the present experiment, not only the protein digestibility of the diet, *per se*, was weak but probably also toxins enhanced endogenous protein losses, explaining why the differential digestibility of jatropha was negative. Moreover, the lower crude fiber digestibility in diets containing jatropha suggests also a negative effect of jatropha on caeca flora.

Finally, the low protein digestibility in the present experiment is probably explained by the weak protein availability of the jatropha kernel meal (Kumar *et al*., 2010) incorporated in corn and the presence of antinutrients which could affect adversely feed utilization. Moreover, despite a growing protein level depending on the incorporation of the jatropha kernel meal, we obtained a differential apparent digestibility coefficient for crude protein which showed no linear change. Indeed, values obtained were lower with 8J and 4J diets, when they were closed to zero with the 12J diet, showing that an additional influence could be exerted on the digestibility of crude protein of jatropha.

Especially as the apparent digestibility coefficient of crude protein in the 12J diet was almost close to that for 4J diet. It could be due to the fact that the jatropha kernel meal acted on the intestinal mucosa resulting in a decrease in viscosity, especially with the 12J diet, more if the amount of the feed intake was very low. This could be the cause of an apparent improvement in the digestibility of crude protein but also ether extract at this level of incorporation. There are, in fact, a negative correlation between viscosity of mucosa in the small intestine and digestibility of fat and crude proteins (White *et al*., 1983; Wang *et al*., 1992). The difference observed also could be incidental, due to the numerous factors taken into account in the final calculation.

This study was the first field experiment of valuation of jatropha kernel seed in poultry feeding in Senegal. The results showed that, despite total dehulling and chemical de-oiling using petroleum ether, jatropha kernel still has a strong negative effect of feed intake and on protein and fiber digestibility. Further studies must be performed in order to assess individual and combined effects of thermal, chemical and biological detoxification processes on jatropha seeds.

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**References**

ABDEL-SHAFY S. and NASR S.M., 2010. Effect of various levels of dietary *Jatropha curcas* seed meal on rabbits infested by the adult ticks of *Hyalomma marginatum marginatum* I. animal performance, anti-tick feeding and haemogram. Tropical Animal Health Production, 43(2), 347-357.

ABDU-AGUYE I. and SANNUSI A., 1986. Acute toxicity studies with *Jatropha curcas* L. Human & Experimental Toxicology, 5(4), 269-274.

ABOU-ARAB A.A. and ABU-SALEM F.M., 2010. Nutritional quality of *Jatropha curcas* seeds and effect of some physical and chemical treatments on their anti-nutritional factors. African Journal of Food Science, 4(3), 93-103.

ACHTEN W., 2010. Sustainability evaluation of biodiesel from *Jatropha curcas* L. A life cycle oriented study. Doctoraatsproefschrift n°921 aan de faculteit Bio-ingenieurswetenschappen van de K.U. Leuven, 176p.

ACHTEN W.M.J, MATHIJS E., VERCHOT L., SINGH V.P., AERTS R. and MUYS B., 2008. Jatropha biodiesel fueling sustainability? Biofuels, Bioproducts and Biorefining, 1(2), 283-291.

ADERIBIGBE A.O., JOHNSON C.O.L.E., MAKKAR H.P.S., BECKER K. and FOIDL N., 1997. Chemical composition and effect of heat on organic matter- and nitrogen-degradability and some antinutritional components of jatropha meal. Animal Feed Science and Technology, 67(2), 223-243.

ADOLF W., OPFERKUCH.J. and HECKER E., 1984. Irritant phorbol derivatives from four jatropha species. Phytochemistry, 23(1), 129-132.

AHMED O.M.M. and ADAM S.E.I., 1979a. Effects of *Jatropha curcas* on calves. Veterinary Pathology, 16(4), 476-482.

AHMED O.M.M. and ADAM S.E.I., 1979b. Toxicity of *Jatropha curcas* in sheep and goats. Research in Veterinary Science, 27(1), 89-96.

AHMED W.A. and SALIMON J., 2009. Phorbol ester as toxic constituents of tropical *Jatropha curcas* seed oil. European Journal of Scientific Research, 31(3), 429-436.

AITKEN A., 1987. The activation of protein kinase C by daphnane, ingenane and tigliane diterpenoid esters. Botanical Journal of the Linnean Society, 9(1&2), 247-263.

ANNONGU A.A., JOSEPH J.K., APATA D.F., ADEYINA A.O., YOUSUF M.B. and OGUNJIMI K.B., 2010. Detoxification of *Jatropha curcas* seeds for use in nutrition of monogastric livestock as alternative feedstuff. Pakistan Journal of Nutrition, 9(9), 902-904.

AOAC, 1990. Official Methods of Analysis (Volume 1). 15$^{th}$Edn. Association of Official Analytic Chemists, Washington DC., USA.

AREGHEORE E.M., BECKER K. and MAKKAR H.P.S., 2003. Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats. The South Pacific Journal of Natural Science, 21(1), 51-56.

BECKER K. and MAKKAR H.P.S, 1998. Effects of phorbol esters in carp (*Cyprinus carpio* L.). Veterinary & Human Toxicology, 40(2), 82-86.

BECKER K. and MAKKAR H.P.S., 2008. *Jatropha curcas*: a potential source for tomorrow's oil and biodiesel. Lipid Technology, 20(5), 104-107.

BEERENS P., 2007. Screw-pressing of Jatropha seeds for fuelling purposes in less developed countries. Msc Dissertation, Department of Sustainable Energy Technology, Eindhoven University of Technology, Eindhoven. 80p.

BELEWU M.A., BELEWU K.Y. and OGUNSOLA F.O., 2010. Nutritive value of dietary fungi treated *Jatropha curcas* kernel cake: voluntary intake, growth and digestibility coefficient of goat. Agriculture and Biology Journal of North America, 1(2), 135-138.

BRONDEAU M.T., FALCY M., JARGOT D., MIRAVAL S., PROTOIS J.C., REYNIER M. and SCHNEIDER O., 1999. Fiche toxicologique n°96. INRS, Cahiers de notes documentaires – Hygiène et sécurité du travail – N°174, 149-153.

CARBONARO M., CAPPELLONI M., NICOLI S., LUCARINI M. and CARNOVALE E., 1997. Solubility-Digestibility relationship of legume proteins. Journal of Agriculture and Food Chemistry, 45(9), 3387-3394.

CHIVANDI E., MTIMUNI J.P., READ J.S. and MAKUZA S.M., 2004. Effects of processing method on phorbol esters concentration, total phenolics, trypsin inhibitor activity and the proximate composition of the Zimbabwean *Jatropha curcas* provenance: a potential livestock feed. Pakistan Journal of Biological Sciences, 7(6), 1001-1005.

DAHOUDA M., TOLEBA S.S., YOUSSAO A.K.I., HAMBUCKERS A., DANDOU-SAPOHO R., MARTIN G.B., FILLET M. and HORNICK J.-L., 2009. Nutrient digestibility of Mucuna (*Mucuna pruriens* var. *utilis*) bean in guinea fowl (*Numida meleagris*, L): effects of heat treatment and levels of incorporation in diets. British Poultry Science, 50(5), 564-572.

DEVAPPA R.K., MAES J., MAKKAR H.P.S. GREYT W.D. and BECKER K., 2010. Quality of biodiesel prepared from phorbol ester extracted *Jatropha curcas* oil. Journal of the American oil Chemists' Society, 87(6), 697-704.

DUKE J.A., 2002. CRC Handbook of Medicinal Herb, 2nd edition. CRC Press, Boca Raton, 870p.

FOIDL N., FOIDL G., SANCHEZ M., MITTELBACH S. and HACKEL S., 1996. *Jatropha curcas* L. as a source for the production of biofuel in Nicaragua. Bioresource Technology, 58(1), 77-82.

FRANCIS G., MAKKAR H.P.S. and BECKER K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199(3-4), 197-227.

GANDHI V.M., CHERIAN K.M. and MULKY M.J., 1995. Toxicological studies on ratanjyot oil. Food and Chemical Toxicology, 33(1), 39-42.

GAUR S., 2009. Development and evaluation of an effective process for the recovery of oil and detoxification of meal from *Jatropha curcas*. Thesis presented to the faculty of the Graduate School of the Missouri University of Science and Technology, Master of Science in chemical engineering, 57p.

HAJOS G., GELENCSER E., PUSZTAI A., GRANT G., SAKHRI M. and BARDOCZ S., 1995. Biological effects and survival of trypsin inhibitors and the agglutinin from soybean in the small intestine of the rat. Journal of Agricultural and Food Chemistry, 43(1), 165-170.

HELLER J., 1996. Promoting the conservation and use of underutilized and neglected crops. 1. Physic nuts *Jatropha curcas* L. International Plant Genetic Resources Institute, Rome, 66p.

HENNING R., 2003. The Jatropha booklet – A guide to the jatropha system and its dissemination in Africa. BaganíGbR.- 37p.

INRA, 1989. L'alimentation des animaux monogastriques: porc, lapin, volailles. (2ème Ed.) INRA Paris, 286p.

JOHNSON L., 2008. Recovery, refining, converting, and stabilizing edible fats and oils. In: Foods Lipids: chemistry, nutrition, and biotechnology. Eds Akoh C.C. and Min D.B., New-York, USA, 206-241

KATOLE S., SAHA S.K., SASTRY V.R.B., LADE M.H. and PRAKASH B., 2011. Intake, blood metabolites and hormonal profile in sheep fed processed jatropha (*Jatropha curcas*) meal. Animal Feed Science and Technology, 170(1-2), 21-26.

KUMAR V., MAKKAR H.P.S. and BECKER K., 2010. Nutritional, physiological and haematological responses in rainbow trout (*Oncorhynchus myskiss*) juveniles fed detoxified *Jatropha curcas* kernel meal. Aquaculture Nutrition, 17(4), 451-467.

LAGO R.C.A., 2009. Castor and jatropha oils: production strategies – a review. Oleagineux, Corps Gras, Lipides, 16(4), 241-247.

LEVINGSTON R. and ZAMORA R., 1983. Medicine trees of the tropics. Unasylva, 35, 7-10.

MAKKAR H.P.S., ADERIBIGBE A.O. and BECKER K., 1998. Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic effects. Food Chemistry, 62(2), 207-215.

MAKKAR H.P.S. and BECKER K., 2009. *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. European Journal of Lipid Science and Technology, 111(8), 773-787.

MAKKAR H.P.S., BECKER K., SPORER F. and WINK M., 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. Journal of Agricultural and Food Chemistry, 45(8), 3152-3157.

MAKKAR H.P.S. and BECKER K., 1999. Nutritional studies on rats and fish (carp *Cyprinus carpio*) fed diets containing unheated and heated *Jatropha curcas* meal of non-toxic provenance. Plants Foods for Human Nutrition, 53(3), 183-192.

MARTINEZ-HERRERA J., Aderibigbe A.O. and Becker K., 1998. Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. Food Chemistry, 62(2), 207-215.

MARTINEZ-HERRERA J., Siddhuraju P., Francis G., Davila-Ortiz G. and Becker K., 2006. Chemical composition, toxic/antimetabolic constituents, and effects if different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. Food Chemistry, 96(1), 80-89.

OLADUNJOYE I.O., OJEDIRAN T., ARINGBANGBA C., AKINRINLADE O.S. and OPAKUNLE O.G., 2014. Effects of inclusion level and length of fermentation on the utilization of jatropha (*Jatropha curcas*) seed cake by broiler chickens. International Journal of Current Microbiology and Applied Sciences, 3(7), 44-54.

RAKSHIT K.D., DARUKESHWARA J., RAJ K.R., NARASIMHAMURTHY K., SAIBABA P. and BHAGYA S., 2008. Toxicity studies of detoxified jatropha meal (*Jatropha curcas*) in rats. Food and Chemical Toxicology, 46(12), 3621-3625.

SIRISHA P., KUMAR A.A., ANJANEYULU Y. and MADHURI D., 2009. Pathological changes in jatropha (*Jatropha curcas*) deoiled seed cake induced toxicity in broiler chicken and its amelioration. Indian Journal of Veterinary Pathology, 33(1), 25-29.

SIVARAMAKRISHNAN S. and GANGADHARAN D., 2009. Edible oil cakes, 253-271, In: P. Singh and A. Pandey (Editors). Biotechnology for Agro-Industrial Residues Utilization, Vol 1. Utilization of agro-residues. Springer, the Netherlands.

STEINFELD H., GERBER P., WASSENAAR T., CASTEL V., ROSALES M. and De HAAN C., 2006. Livestock's long shadow – Environmental issues and options. Food and Agriculture Organization of the United Nations: Rome, Italy.

STIRPE F., PESSION-BRIZZI A., LORENZONI E., STROCCHI P., MONTANARO L. and SPERTI S., 1976. Studies on the proteins from the seeds of *Croton tiglium* and of *Jatropha curcas*. Biochemical Journal, 159, 1-6.

TEMLER R.S., DORMOND C.A. and FINOT P.A., 1983. Biological assessment of ptoteins from different sources by protein efficiency ratio (per) and by nitrogen retention. Nutrition Reports International, 28(2), 267-276.

VAKNIN Y., GHANIM M., SAMRA S., DVASH L., HENDELSMAN E., EISIKOWITCH D.and SAMOCHA Y., 2011. Predicting *Jatropha curcas* seed-oil content, oil composition and protein content using near-infrared spectroscopy-a quick and non-destructive method. Industrial Crops and Products, 34, 1029-1034.

WANG L., NEWMAN R.K., NEWMAN C.W. and HOFER P.J., 1992. Barley β-glucan alter intestinal viscosity and reduce plasma cholesterol concentrations in chicks. Journal of Nutrition, 122, 2292-2297.

WHITE C.E., CAMPBELL D.R. and McDOWELL L.R., 2000. Effects of dry matter on trypsin inhibitors and urease activity in heat treated soya beans fed to weaned piglets. Animal Feed Science and Technology, 87(1-2), 105-115.

WHITE L., BIRD H.R., SUNDE M.L. and MARLETT J.A., 1983. Viscosity of β-glucan as a factor in the enzymatic improvement of barley for chicks. Poultry Science, 62, 853-862.

XIAO J., ZHANG H., NIU L., WANG X. and LU X., 2011. Evaluation of detoxification methods on toxic and antinutritional composition and nutritional quality of proteins in *Jatropha curcas* meal. Journal of Agricultural and Food Chemistry, 59(8), 4040-4044.

ZHENG Y.I., WIESENBORN D.P., TOSTENSON K., and KANGAS N., 2003. Screw pressing of whole and dehulled flaxseed for organic oil. Journal of the American Oil Chemists' Society, 80(10), 1039-2045.

## CHAPITRE III

**Effets de l'incorporation de l'amande de *Jatropha curcas* ayant subi un déshuilage chimique et un traitement thermique dans l'alimentation de poulets de chair en production**

**Effects of defatting combined or not to heating of *Jatropha curcas* kernel meal on feed intake and growth performance in broiler chickens and chicks in Senegal**

**Article 4 – Soumis et accepté pour publication par la revue Tropicultura**

Thierry Daniel Tamsir NESSEIM, Abdoulaye DIENG, Guy MERGEAI & Jean-Luc HORNICK

**Abstract**

*Jatropha curcas* is a tropical plant belonging to the *Euphorbiaceae* family whose cultivation has been largely promoted in recent years for the production of biofuels. The kernel of the seed contains approximately 55% lipid in dry matter and the meal obtained could be an exceptional source of protein for family poultry farming, after treatments to remove toxic and anti-nutritional compounds. The ingestion and the growth performance of *J. curcas* Kernel Meal (JKM), obtained after partial physico chemical de-oiling combined or not with heating was evaluated in broiler chickens and chicks.

Sixty unsexed broiler chickens, 30 day-old, divided into three groups as well as twenty broiler chicks, 1 day-old, divided into two groups were obtained for two experiments.

In experiment 1, jatropha kernel was de-oiled and incorporated into a control fattening ($0JKM_1$) feed at 40 and 80g/kg (diets $4JKM_1$ and $8JKM_1$). In experiment 2, jatropha kernel meal obtained in experiment 1 was heat treated and incorporated into a growing diet at 80g/kg (diet $8JKM_2$).

Daily dietary intakes as well as weight gain of the animals were affected by the incorporation of jatropha kernel meal in the ration. In experiment 1, Average Daily Feed Intake ($ADFI_1$) of 139.2, 55.2 and 23.4g/day/animal and also Average Daily Weight Gain ($ADWG_1$) of 61.9, 18.5 and -7.7g/animal were obtained respectively for the groups fed with diets $0JKM_1$, $4JKM_1$ and $8JKM_1$. In experiment 2, Average Daily Feed Intake ($ADFI_2$) of 18.7 and 3.1g/day/animal and also Average Daily Weight Gain ($ADWG_2$) of 7.1 and 1.9g/animal were obtained respectively for the groups fed with diets $0JKM_2$ and $8JKM_2$.

In both experiment, Feed Conversion Ratio (FCR) was also affected by the dietary treatments and the overall mortality rate showed an increase according to levels of jatropha kernel meal in diet.

**Keywords:** broiler chickens, broiler chicks, *Jatropha curcas*, ingestion, growth performance

**Titre**

Effets d'une délipidation combinée ou non au chauffage d'un tourteau d'amande *Jatropha curcas* sur l'ingestion alimentaire et les performances de croissance de poulets et poussins de chair au Sénégal

**Titre abrégé**

Tourteau d'amande de *Jatropha curcas* en alimentation des volailles

**Résumé**

*Jatropha curcas* est une plante appartenant à la famille des *Euphorbiaceae* dont la culture a été largement promue au cours des dernières années pour la production de biocarburants. Sa graine referme une amande qui contient environ 55% de matière grasse par rapport à la matière sèche et le tourteau obtenu après l'extraction de l'huile pourrait être une source exceptionnelle de protéine notamment en aviculture familiale, après des traitements destinés à supprimer les composés toxiques et antinutritionnels. L'ingestion et les performances de croissance du tourteau de l'amande de *J. curcas*, obtenu après déshuilage physico-chimique partiel et traitement à la chaleur, ont été évaluées sur des poulets en croissance et des poussins de chair.

Soixante poulets de chair, âgés de 30 jours, divisés en trois groupes ainsi que 20 poussins de chair, âgés d'un jour, divisés en deux groupes ont été obtenus pour deux expériences.

Dans l'expérience 1, de l'amande de jatropha a été déshuilée et incorporée à un aliment témoin ($0JKM_1$) à raison de 40 et 80g /kg (rations $4JKM_1$ et $8JKM_1$). En expérience 2, le produit déshuilé obtenu en expérience 1 a subi un traitement thermique puis a été incorporé dans un aliment de démarrage à raison de 80g/kg (ration $8JKM_2$).

L'ingestion moyenne quotidienne ainsi que la croissance pondérale ont été affectées par l'incorporation du tourteau d'amande de jatropha dans la ration. En expérience 1, les animaux ont présenté des ingestions moyennes de 139,2 ; 55,2 et 23,4g/jour/animal ainsi que des gains de poids moyens quotidiens de 61,9 ; 18,5 et -7,7g/animal avec, respectivement, les rations $0JKM_1$, $4JKM_1$ et $8JKM_1$. En expérience 2, des ingestions moyennes quotidiennes de 18,7 et de 3,1g/jour/animal tandis que des gains de poids moyens quotidiens de 5,9 et 1,7g/animal ont été obtenus respectivement pour les groupes nourris avec des régimes $0JKM_2$ et $8JKM_2$.

Dans les deux expériences, l'indice de consommation a aussi été affecté par le niveau d'incorporation du jatropha et il en est de même pour le taux de mortalité total enregistré.

**Mots clés:** poulets de chair, poussins de chair, *Jatropha curcas*, ingestion, performance de croissance

## 1. Introduction

To meet the population demand for animal proteins and lower the cost production, including those of poultry, expenses related to feed must be reduced. In Senegal, many raw materials and agricultural by-products used in the manufacture of feed are available (12). However, oilseeds meal used in poultry feed are mainly those of peanut and cotton. This is due to low domestic soybean meal production and high cost of imports thereof. A challenge is to find out alternatives valuing plant resources that abound in the country. Some by-products of these resources contain anti-nutritional or toxic natural substances that limit their use. Potentially usable byproduct is cake of *Jatropha curcas* seed. The plant, known as physic nut, or *tabanani* in Senegal, is a wild drought-resistant shrub belonging to the *Euphorbiaceae* family, which can grow in marginal wastelands and planted as a fence to protect fields because it is not consumed by animals (25). The oil content of the seed is about 22 to 48% (9), can be converted into bio-diesel (14). The kernel meal, obtained after shelling the seed and oil extraction, contains about half protein (4). It is nitrogen-rich and a very good soil fertilizer (19), but its use in animal feed remains limited. The seed was found to be toxic for several species types such as rat and rabbit (16), chicks (13) and also fish (8).

Jatropha toxicity is due to diterpene derivatives classified as phorbol esters (29). They activate protein kinase C, an enzyme which plays an essential role, regulating cell growth and differentiation (11). In addition, the nut by-products contain curcin, a lectin which has an effect on the protein synthesis (26) and anti-nutritional factors such as trypsin inhibitors, saponins and phytate which interfere with digestive process in animals (4). Particularly in chickens, toxicity manifested as growth depression was observed with unprocessed meal (35). The cake should be detoxified before being use as feed. Common detoxification methods are essentially chemical (8, 10), e.g., de-oiling. Heating can give additional interest.

The objective of this study was to evaluate in Senegal, the impact of the introduction in a diet of *J. curcas* kernel meal physico-chemically de-oiled or de-oiled and heated, on ingestion and growth performance of broiler chickens.

## 2. Materials and methods

### 2.1. Location of the experiment

Experiments were conducted in *Ecole Nationale Supérieure d'Agriculture* (*ENSA*), University of Thies (Senegal) in two stages. The first part (experiment 1) was planned during the dry season (April) with a temperature ranging from 21.9 to 34.7°C, a relative humidity ranging from 22.4 to 53.4% and the second part (experiment 2) just after the rainy season (October) with a temperature ranging from 24.8 to 34.7°C and a relative humidity ranging from 59.3 to 96.5%.

### 2.2. Preparation of the jatropha kernel meal and diet formulation

Thirty kg mature and dry seeds of *J. curcas* were collected from Dialacoto, Senegal. The seeds were cracked and unshelled manually to obtain kernels, which were grinded to get a Jatropha Kernel Paste (JKP). A residual level of Ether Extract (EE) lower than 100 grams per kilo gram (g/kg) Dry Matter (DM) was judged to be adequate to perform the trial. Oil extraction with petroleum ether (boiling range 60-80°C) was assessed diluting 3vol ether in 1vol JKP, assuming a homogeneous distribution of the solvent in the mass. The JKP was introduced into a barrel and dipped in petroleum ether (1:3vol.) for 7 consecutive days. Meanwhile, the drum was regularly shaken to allow the kernel impregnation with ether. At the end of the soaking process, the kernel was recovered by filtering oil and ether and drying in the sun. Defatted Jatropha Kernel Meal (JKM) was obtained.

Experiment 1: JKM was incorporated as such in a commercial feed (SEDIMA S.A.) at levels of 40 and 80g/kg (diets $4JKM_1$ and $8JKM_1$) (**Table 1**). This commercial feed, constituting the control diet ($0JKM_1$), was mainly composed of maize, cereals issues, soybean meal, peanut meal, fish meal, calcium carbonate and vitamin-mineral complex. Extra-maize warranted iso-nitrogenous and iso-caloric traits of the diets.

Experiment 2: JKM was placed in a drying oven (105°C for 2 hours), cooled and then incorporated at 80g/kg in an experimental diet ($0JKM_2$) at 80g/kg (diet $8JKM_2$), in substitution of groundnut cake (**Table 2**), allowing iso-nitrogenous and iso-caloric properties of the diets.

### 2.3. Animals and housing

Experiment 1: sixty unsexed broiler chickens, 30 days-old, local strain Ross 308, were obtained, divided into three groups of twenty subjects corresponding to the three dietary treatments ($0JKM_1$, $4JKM_1$ and $8JKM_1$) and maintained during their final growth phase. The experiment was carried out for 15 days from the day 30.

Experiment 2: twenty unsexed one-day old chicks, local strain Ross 308, were obtained, divided into two groups of ten subjects corresponding to the two dietary ($0JKM_2$ and $8JKM_2$) treatments and maintained during their growing phase. The experiment was carried out for 15 days from the day 1.

Animals were kept in a well-ventilated broiler chickens barn in which three areas, separated by fences of 0.75m in height, were installed. Each area, which was 4.5m² (3x1.5m) and was provided with troughs suitable for the distribution of feed and water.

During the test, water was available *ad libitum*. Feed was weighed early in the morning and provided once a day. Refusals of food were collected and weighed the day after the distribution. Animals were kept on a concrete floor that was previously disinfected.

### 2.4. Feed consumption and growth performance

Every day, the amounts of feed distributed and the rejected quantities of the previous day were recorded to determine the feed intake by animal. The daily group amounts of feed intake = feed supplies – feed rejected, were recorded. It was deduced the Average Daily Feed Intake (ADFI).

The Feed Conversion Ratio (FCR) was determined as the feed intake per unit weight gain.

During experiments, birds in each replicate were individually weighed at the beginning of the experiment and weekly thereafter to monitor the growth. Weight gain was determined as the difference in weights between two successive weeks. It was deduced the Average Daily Weight Gain (ADWG).

Mortality in each replicate was calculated as the percentage of the total number of birds in the replicate at the beginning of the experiment.

### 2.5. Chemical analyses

At the beginning of the experiment, samples of the raw materials but also of the diets were collected for analysis.

Chemical analyses were performed according to the procedures of AOAC (6). Crude protein (CP) was determined by the Kjeldahl method (N x 6.25), ether extract (EE) by the Soxhlet method and Crude fiber (CF) by the method of Weende.

The following values were calculated from those measured:

Organic Matter (OM) = 100 – Ash

Nitrogen Free Extract (NFE) = OM – EE – CP – CF

The true Metabolic Energy of each diet is given by the following formula: ME (kcal/kg DM) = 3951 + (54.4 x EE) – (88.7 x CF) – (40.8 x Ash) (36).

### 2.6. Statistical analysis

All data generated were subjected to analysis of Variance in a Complete Randomized Design of Statistix 8.1 software package. Significant means were separated using Tukey HSD all-pairwise comparisons test of the same package. Mortalities data were compared according to Fischer's Exact test.

## 3. Results

### 3.1. Chemical composition of feed

Effect of various processing methods on proximate and energy composition of *Jatropha curcas* Kernel (JK), *J. curcas* Kernel Meal (JKM) and experimental diets are shown in **Table 3**.

Kernel contained 560g EE/kg Dry Matter (DM) and about 260g CP/kg DM, while Ash remained close to 50g/kg DM. Crude Fiber and Nitrogen Free Extract (NFE) reached values, respectively of 120 and 24g/kg DM. The residual level of EE in jatropha kernel meal almost reached the objective of 100g/kg DM. As a consequence, CP represented about half of material. NFE represented about a quarter of DM, while CF and ash remained close to 100g/kg DM.

For experiment 1, the control feed, which was the carrier of the diets, gave a crude protein level of 220g/kg DM. Ether extract and CF remained below 100g/kg DM, while ash were 180g/kg DM. The NFE constituted about half of the dry matter. Diets ($4JKM_1$ and $8JKM_1$) that were used for this experiment were developed so as to provide protein and energy levels approximately similar. Moreover, the other nutrients remained in the same values for all diets. The value of the real metabolic

energy was calculated to be about 3200kcal/kg DM for the control diet. Concerning diets 4JM and 8JM, this value was calculated, respectively, to about 3300 and 3200kcal/kg DM.

For experiment 2, diets ($0JKM_2$ and $8JKM_2$), showed almost similar values with regard to the DM, CP, ash and Metabolic Energy (ME) which were respectively 900g/kg DM, 20g/kg DM, 11g/kg DM and 3500kcal/kg DM. Only the EE and CF were slightly different (respectively 120 vs. 104g/kg DM and 66 vs. 58g/kg DM).

Analytical results showed that diets were iso-protein and iso-energetic.

### 3.2. Feed consumption

**Table 4** shows the daily individual feed intake by animals during the experiments. It was inversely proportional to the incorporation of jatropha kernel meal and significantly different ($P<0.05$) in the first and in the second experiment. In experiment 1, for animals fed with $0JKM_1$, the $ADFI_1$ was 139.2±13.3g/d/an (gram per day per animal) while it was 55.2±27.5 and 23.4±24g/d/an, respectively, for $4JKM_1$ and $8JKM_1$. In experiment 2, the $ADFI_2$ of animals fed with $0JKM_2$ was 18.7±4.9g/d/animal when it was 3.1±2.5g/d/animal for $8JKM_2$. The FCR presented mean values that varied from 2.3 for $0JKM_1$, to 17.4 for $4JKM_1$ and -0.5 for $8JKM_1$, and values that varied between 3.1 and 1.9, respectively for $0JKM_2$ and $8JKM_2$ , without significant difference ($P>0.05$).

### 3.3. Growth performance

During the two weeks, it was found that the control group showed a linear weight growth, evolving from 1153.5±148.7g on day 30 (d30) to 2078.1±360.1g on day 44 (d44). For the same periods, animals that received the $4JKM_1$ diet showed lower performance, from 1132.1±267.8g to 1407.5±211.1g. Finally, the animals that received the $8JKM_1$ diet, in turn, presented a decreasing weight change over the weights from 1200.6±110.1g to 1089.6±124.4g. Thus, average daily weight gain (ADWG) per animal significant ($P<0.05$) evolved inversely to the incorporation of jatropha kernel meal, ranging from 61.9g/d/animal for the control group to 18.5g/d/animal for the $4JKM_1$ group and -7.7g/d/animal for the $8JKM_1$ group (**Table 4**).

The total mortalities recorded during experiment 1 showed an increase without significant difference ($P>0.05$) according the incorporation of jatropha kernel meal in diets, from 0% for the control group to 5 and 20% for respectively the $4JKM_1$ and $8JKM_1$ groups.

In experiment 2, it was found that the control group showed a linear weight increase, evolving from 45.7±2.2g on day 1 (d1) to 135.5±29.8g on day 15 (d15). For the same periods, animals that received the 8JKM2 diet showed lower weight, from 46.1±2.9g to 70.9±24.2g. Thus, average daily weight gain (ADWG) per animal had evolved inversely to the incorporation of jatropha kernel meal, and without significant ($P>0.05$), ranging from 7.1g/d/animal for the control group to 1.9g/d/animal for $8JKM_2$ group.

During experiment 2, the total mortalities recorded showed a significant difference ($P<0.05$) according incorporation of the jatropha kernel meal in diets, from 0% for the control group to 60% for the $8JKM_2$ group.

## 4. Discussion

The extract of vegetable oil from seeds is mainly based on two processes which are mechanical pressing and solvent extraction. Mechanical screw press is a mean of oilseed crushing to small and medium scale (40). To avoid the presence of low digestible shells in monogastrics (20), and thus to study the specific effects of kernels on poultry, seeds were shelled manually. After shell removing, the kernels contained between approximately 550g/kg in DM of EE. These values confirm those obtained by (4), (24), (27), (30) and those compiled by (3).

Use of mechanical press for de-oiling the kernel after shelling did not allow a satisfactory extraction of oil and just helped to reduce it into a paste. To overcome this difficulty, petroleum ether was used to allow oil extraction from the paste. The solvent is a special gasoline G type, colorless liquid, of low viscosity and very good solvent of greases. It allows direct extraction by exhaustion. However, its flammability, toxicity and price indexed to oil prices are major disadvantages of its use (22).

The de-oiling process used yielded about 10% residual fat in the dry matter of jatropha kernel meal. These values matched those obtained by (4) with a partial de-oiling with a screw press, but higher than those obtained by the same authors and (27) with a totally defatted meal. These differences in results compared to the method used, can be explained by the process of de-oiling. Indeed, the last authors implemented a soxhlet de-oiling which eliminates all the fat of the matter. Soaking method used presently, more compatible with a field experiment, left a significant amount of fat. Concerning the procedure that was used, the kernel paste was dipping in petroleum ether (1:3vol.) inside a barrel

where it was maintained for 7 successive days and then was recovered by draining residual ether and drying in the sun to allow the ether evaporation.

For experiment 2, following the de-oiling process, the JKM powder obtained in the first experiment was treated by passage in a drying oven (105°C for 2 hours). The aim of this stage was to inactivate toxic and anti-nutritional compounds. Indeed, (31), by heat treatment in an autoclave (121°C for 20mn), significantly inactivated trypsin inhibitor activities which are anti-nutritional factors but also lectin activity which is considered to be another toxic factor in *J. curcas* seeds. In the same way, (2) decreased the concentration of trypsin inhibitor and lectin by about 75 and 83% respectively. These results were in agreement with (18) who reported also that heat treatment has a positive effect on reducing trypsine inhibitor and lectine concentration in *J. curcas* meal.

Chemical analyzes made on JKM showed that it mainly consist of crude protein and nitrogen free extract (723g/kg in DM). The levels of crude protein and ash were similar with those obtained by (4) for partially de-oiled cake, but the values in EE and especially in crude fiber were higher.

Jatropha meal showed good nutritional potential with a level of crude protein noted higher than that of soybean meal (27), confirming the protein concentrate nature of this product for poultry feed. Diets offered during the experimentation showed a crude protein content of 21% DM. These values corresponded to the recommended ones for broiler chickens production (21).

In our study, the daily intake per broiler chickens and chicks was inversely dependent on the incorporation of the JKM, resulting in lower weight gain, especially for animals that received the 8JKM diet.

The decrease feed intake recorded during experimental sequence, which was reflected in weight gain, was probably related to the incorporation of the jatropha kernel meal in diet. Feed intake was influenced by a variety of factors, such as taste, smell and texture of the diet (38). The decline was probably related of palatability as animals systematically reduced their consumption whenever they were exposed to jatropha. The daily feed intake and body weight change during the test sequence were significantly lower in jatropha kernel groups in comparison with control group. These results confirmed those obtained by (37) which incorporated *J. curcas* meal at the level of 5% in the diets of broiler chickens and observed reduced feed intake. This shows the negative effect on feed palatability

of the jatropha meal. To some extent, this could be surprising since investigators did not perceived abnormal taste of the JK. It is possible that discomfort could be perceived by the animal once the product is ingested.

Phorbol esters, the main toxic compounds in *J. curcas* seeds (29), were reported to possess a diterpene named 12-deoxy-16-hydroxyphorbol (17). They were found to be responsible of an irritant effect after topical application, but also caused diarrhea and mortality in the animals (16). These compounds are thermo stable and isolated from the oil of jatropha (7). Chemical de-oiling of jatropha kernel, followed by a physicochemical detoxification treatment does not cause a complete removal of toxic factors including phorbol esters (24). Its inclusion in diets thus gives rise to a decrease in feed intake and a weight gain reduction on monogastrics and the presence of phorbol esters in feed has significant effects on its' acceptance (7). In spite of chemical treatments (sodium chloride and calcium hydroxide) that decreased phorbol esters and haemagglutination activity, (23) observed a reduced nutrient intake. Also, (5) showed a tolerance in diets containing physico chemically treated jatropha kernel meal up to 15%. In addition, mortalities were mainly recorded in experimental group. Monogastrics show great sensibility to this compound in feed (8). They show intestinal irritation and thus feed rejection due to the residual effects of the toxins.

The low animal weight performance observed for group receiving jatropha kernel meal during experiments was probably due to both reduced intake but also poor protein utilization (32). In this respect, trypsin inhibitors and curcin are known to decrease the weight gain performance of animals (15) and were probably related to the level of jatropha kernel in the diet. Tryspin inhibitors are anti-nutritional factors which interfere with the physiological process of digestion in non-ruminants, leading to severe growth depression (39). A similar growth depression due to residual anti-nutritional factors with JKM roasted was observed (34). Furthermore, Other Studies however showed that the feed intake and mortality of animals were not affected by inclusion of jatropha kermel meal fermented with *Aspergillus niger* in their diet, despite a poor feed conversion and a low weight gain (33).

For defatted and untreated jatropha kernel meal, (4) measured a trypsin inhibitor activity to about 20 mg/g of sample. Heat treatments reduced this activity to 0.2 mg/g of sample, showing the thermo labile character of the toxin. The effect of heat treatment was confirmed by (1). In experiment 1, the

kernel of jatropha was de-oiled without heat treatment. Trypsin inhibitors remained present and probably contributed to interference with the physiological digestive process in poultry. These observations are in agreement with those made by (24), (27) and (28) who showed adverse physiological effects in monogastric and therefore a decrease in voluntary intake and reduced weight gain for animals subjected to diets with unheated jatropha kernel meal. Finally, these observations confirm those of our previous studies (32). The growth depression and the poor feed conversion ratio observed for diets incorporating JKM can be attributed to residual anti-nutritional factors like phorbol esters, curcin and trypsin inhibitors that have been reported to be present in jatropha seed.

In experiment 2, the JKM was heat treated. Curcin and Trypsin inhibitors were presumed to be removed eliminated but this did not improve feed intake of animals and thus weight gain. This confirms observations of (35) which showed that chemical followed by heat treatments allowed the removal of curcin and some anti-nutritionnal factors such as trypsin inhibitors. However, the reactions of animals that consumed the tested feed let thinking that there was still a toxic compound in the JKM. This was probably a significant fraction of phorbol esters which was not eliminated.

This study was the first field experiment on evaluation of jatropha kernel seed in broiler chickens and chicks production in Senegal. The results showed that, despite total dehulling, chemical de-oiling using petroleum ether and heat treatment, jatropha kernel meal still conserve a strong negative effect on feed intake and then on growth performance despite the short period of incorporation. Further studies must be performed in order to assess combined effects of thermal, chemical and biological detoxification processes on jatropha seeds.

**References**


1. ABOU-ARAB A.A. & ABU-SALEM F.M., 2010, Nutritional quality of *Jatropha curcas* seeds and effect of some physical and chemical treatments on their anti-nutritional factors. Afr. J. Food Sci., **4**, 3, 93-103.
2. ABO El-FADEL M.H., Hussein A.M. & Mohamed A.H., 2011, Incorporation *Jatropha curcas* meal on lambs ration and it's effect on lambs performance. J. Am. Sci., **7**, 2, 129-132.

3. ACHTEN W., 2010, Sustainability evaluation of biodiesel from *Jatropha curcas* L. A life cycle oriented study. Doctoraatsproefschrift n°921 aan de faculteit Bio-ingenieurswetenschappen van de K.U. Leuven, 176p.
4. ADERIBIGBE A.O., JOHNSON C.O.L.E., MAKKAR H.P.S., BECKER K. & FOIDL N., 1997, Chemical composition and effect of heat on organic matter- and nitrogen-degradability and some antinutritional components of jatropha meal. Anim. Feed Sci. Technol., **67**, 2, 223-243.
5. ANNONGU A.A., JOSEPH J.K., APATA D.F., ADEYINA A.O., YOUSUF M.B. & OGUNJIMI K.B., 2010, Detoxification of *Jatropha curcas* seeds for use in nutrition of monogastric livestock as alternative feedstuff. Pakistan J. Nutrition, **9**, 9, 902-904.
6. AOAC, 1990, Official Methods of Analysis (Volume 1). 15$^{th}$Edn. Association of Official Analytic Chemists, Washington DC., USA.
7. AREGHEORE E.M., BECKER K. & MAKKAR H.P.S., 2003, Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats. The S. Pac. J. Nat. Appl. Sci., **21**, 1, 51-56.
8. BECKER K. & MAKKAR H.P.S, 1998, Effects of phorbol esters in carp (*Cyprinus carpio* L.). Vet. Hum. Toxicol., **40**, 2, 82-86.
9. BECKER K. & MAKKAR H.P.S., 2008, *Jatropha curcas*: a potential source for tomorrow's oil and biodiesel. Lipid Technol., **20**, 5, 104-107.
10. CHIVANDI E., MTIMUNI J.P., READ J.S. & MAKUZA S.M., 2004, Effects of processing method on phorbol esters concentration, total phenolics, trypsin inhibitor activity and the proximate composition of the Zimbabwean *Jatropha curcas* provenance: a potential livestock feed. Pak. J. Biol. Sci., **7**, 6, 1001-1005.
11. DEMPSEY E.C., NEWTON A.C., MOCHLY-ROSEN D., FIEDLS A.P., REYLAND M.E., INSEL P.A. & MESSING R.O., 2000, Protein kinase C isozymes and the regulation of diverse cell responses. Am. J. Physiol. – Lung C., **279**, 3, 429-438.
12. DIEYE P.N., MISSOHOU A. & FAYE A., 2010, L'aviculture familiale: un levier pour améliorer les revenus des éleveurs pauvres au sud du Sénégal. In: Faye B. and Duteutre G. (Ed.): L'élevage, richesse des pauvres, Paris: Editions Quae, 191-201.

13. El BADWI S.M., ADAM S.E. & HAPKE H.J., 1995. Comparative toxicity of *Ricinus communis* and *Jatropha curcas* in Brown Hisex chicks. Dtsch. Tierarztl. Wochenschr., **102**, 2, 75-77.
14. FOIDL N., FOIDL G., SANCHEZ M., MITTLEBACH S. & HACKEL S., 1996, *Jatropha curcas* L. as a source for the production of biofuel in Nicaragua. Bioresource Technol., **58**, 1, 77-82.
15. FRANCIS G., MAKKAR H.P.S. & BECKER K., 2001, Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, **199**, 3-4, 197-227.
16. GANDHI V.M., CHERIAN K.M. & MULKY M.J., 1995, Toxicological studies on ratanjyot oil. Food Chem. Toxicol., **33**, 1, 39-42.
17. HAAS, W., STERK H., & M. MITTLEBACH, 2002, Novel 12-Deoxy-16-hydroxyphorbol diesters isolated from the seed oil of *Jatropha curcas*. J. Nat. Prod., **65**, 10, 1734-1440.
18. HAAS W. & MITTLEBACH M., 2000, Detoxification experiments with the seed oil from *Jatropha curcas* L. Ind. Crop. Prod., **12**, 2, 111-118.
19. HELLER J., 1996, Promoting the conservation and use of underutilized and neglected crops. 1. Physic nuts *Jatropha curcas* L. International Plant Genetic Resources Institute, Rome, 66p.
20. HUISMAN J. & TOLMAN G.H., 1992, Antinutritional factors in the plant proteins of diets for non-ruminants. In : Garnsworthy P.C., Haresign W. and Cole D.J.A. : Recent advances in animal nutrition, Butterworth-Heinemann Ltd, Oxford, 3-31.
21. INRA, 1989, L'alimentation des animaux monogastriques: porc, lapin, volailles. (2ème Ed.) INRA Paris, 286p.
22. JOHNSON L., 2008, Recovery, refining, converting, and stabilizing edible fats and oils. In: Foods Lipids: chemistry, nutrition, and biotechnology. Eds Akoh C.C. and Min D.B., New-York, USA, 206-241.
23. KATOLE S., SAHA S.K., SASTRY V.R.B., LADE M.H. & PRAKASH B., 2011, Intake, blood metabolites and hormonal profile in sheep fed processed jatropha (*Jatropha curcas*) meal. Anim. Feed Sci. Tech., **170**, 1-2, 21-26.
24. KUMAR V., MAKKAR H.P.S. & BECKER K., 2010, Nutritional, physiological and haematological responses in rainbow trout (*Oncorhynchus myskiss*) juveniles fed detoxified *Jatropha curcas* kernel meal. Aquacult. Nutr., **17**, 4, 451-467.

25. KUMAR A. & SHARMA S., 2008, An evaluation of multipurpose oil seed crop for industrial use (*Jatropha curcas* L.): A review. Ind. Crop. Prod., **28**, 1, 1-10.
26. LIN J., CHEN Y., XU Y., YAN F., TANG L. & CHEN F., 2003, Cloning and expression of curcin, a ribosome-inactivating protein from the seeds of *Jatropha curcas*. Acta Bot. Sin., **45**, 7, 858-863.
27. MAKKAR H.P.S., ADERIBIGBE A.O. & BECKER K., 1998, Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic effects. Food Chem., **62**, 2, 207-215.
28. MAKKAR H.P.S. & BECKER K., 2009, *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. Eur. J. Lipid Sci. Tech., **111**, 8, 773-787.
29. MAKKAR H.P.S., BECKER K., SPORER F. & WINK M., 1997, Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. J. Agr. Food Chem., **45**, 8, 3152-3157.
30. MARTINEZ-HERRERA J., ADEROBIGBE A.O. & BECKER K., 1998, Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. Food Chem., **62**, 2, 207-215.
31. MARTINEZ-HERRERA J., SIDDHURAJU P., FRANCIS G., DAVILA-ORTIZ G. & BECKER K., 2006, Chemical composition, toxic/antimetabolic constituents, and effects if different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. Food Chem., **9**6, 1, 80-89.
32. NESSEIM T.D.T., DIENG A., MERGEAI G., NDIAYE S. & HORNICK J.-L., 2015, Digestibility of solvent-treated *Jatropha curcas* kernel by broiler chickens in Senegal. Trop. Anim. Health Prod., **47**, 8, 1553-1590
33. OLADUNJOYE I.O., OJEDIRAN T., ARINGBANGBA C., AKINRINLADE O.S. & OPAKUNLE O.G., 2014, Effects of inclusion level and length of fermentation on the utilization of jatropha (*Jatropha curcas*) seed cake by broiler chickens. Int. J. Curr. Microbiol. Appl. Sci., **3**, 7, 44-54.

34. OJEDIRAN T.K., ADISA Y.A., YUSUF S.A. & EMIOLA I.A., 2014, Nutritional evaluation of processed *Jatropha curcas* kernel meals: effects on growth performance of broiler chicks. J. Anim. Sci. Adv., **1**, 11, 1110-1121.
35. PASARIBU T., WINA E., TANGENDJAJA B. & ISKANDAR S., 2009. Performance of broiler chicken fed physically and chemically treated jatropha (*Jatropha curcas*) seed meal. Indonesian J. Anim. Vet. Sci., **14**, 1, 11-18.
36. SIBBALD, I.R., 1976, The true metabolizable energy values of several feedingstuffs measured with roosters, laying hens, turkeys and broiler hens. Poultry Science, 55(4), 1459-1463.
37. SUMIATI A., SUDARMAN I.N., HIDAYA I. & SANTOSO W.B., 2007, Toksisitas racun bungkil biji jarak pagar (*Jatropha curcas* L.) pada ayam broiler (toxicity of *Jatropha curcas* L. mealtoxins in the broilers). Proceeding Seminar Nasional AINI VI. Bagian Nutrisid Makanan Ternak, Fakultas Peter nakan, Universities Gadjah Mada. Yogyakarka, Indonesia, 144-150
38. TEMLER R.S., DORMOND C.A. & FINOT P.A., 1983, Biological assessment of ptoteins from different sources by protein efficiency ratio (per) and by nitrogen retention. Nutr. Rep. Int., **28**, 2, 267-276.
39. WHITE C.E., CAMPBELL D.R. & McDOWELL L.R., 2000, Effects of dry matter on trypsin inhibitors and urease activity in heat treated soya beans fed to weaned piglets. Anim. Feed Sci. Tech., **87**, 1-2, 105-115.
40. ZHENG Y.I., WIESENBORN D.P., TOSTENSON K., & KANGAS N., 2003, Screw pressing of whole and dehulled flaxseed for organic oil. J. Am. Oil Chem. Soc., **80**, 10, 1039-2045.

**Table 1:** Composition of diets incorporating the *J. curcas* kernel meal in experiment 1

| Raw materials | $4JKM_1$ | $8JKM_1$ |
|---|---|---|
| | % | % |
| Control diet ($0JKM_1$) | 76.6 | 67.0 |
| JKM | 4.0 | 8.0 |
| Maize | 19.4 | 25.0 |
| **Total** | **100.0** | **100.0** |

JKM = *Jatropha curcas* Kernel Meal, $4JKM_1$ and $8JKM_1$ = control diet (maize, cereals issues, soybean meal, peanut meal, fish meal, calcium carbonate and vitamin-mineral complex) incorporated with 4 and 8% jatropha kernel meal and maize.

**Table 2:** Gross composition of experimental diets for broiler chicks' starters in experiment 2

| Ingredients (%) | $0JKM_2$ | $8JKM_2$ |
|---|---|---|
| Maize | 40.0 | 40.0 |
| Millet | 17.0 | 17.0 |
| Groundnut cake | 23.0 | 15.0 |
| JKM | 0.0 | 8.0 |
| Fishmeal | 10.0 | 10.0 |
| Chalk | 0.5 | 0.5 |
| TCP | 0.3 | 0.3 |
| Peanut oil | 4.5 | 4.5 |
| Synthetic lysine | 0.2 | 0.2 |
| Synthetic methionine | 0.1 | 0.1 |
| Vitamin-mineral Premix | 4.5 | 4.5 |
| **Total** | **100.0** | **100.0** |
| **Calculated values** | | |
| Crude protein (% in DM) | 23.1 | 23.5 |
| ME (kcal/kg DM) | 3130.6 | 3181.2 |

TCP = Tricalcium phosphate, ME = Metabolic Energy, DM = Dry Matter,

$0JKM_2$ = control diet, $8JKM_2$ = diet incorporating 8% of JKM.

**Table 3:** Proximate composition of raw materials and feed used in experiments 1 and 2.

| | DM | Chemical composition (% in DM) | | | | | | ME |
|---|---|---|---|---|---|---|---|---|
| | (%) | OM | CP | EE | CF | Ash | NFE | (kcal/kg DM) |
| JK | 96.1 | 95.2 | 25.9 | 55.5 | 11.6 | 4.8 | 2.4 | 5749.4 |
| JKM | 95.0 | 90.7 | 48.6 | 10.0 | 6.5 | 9.4 | 25.6 | 3537.4 |
| $0JKM_1$ | 90.7 | 81.8 | 22.0 | 7.5 | 5.1 | 18.2 | 47.2 | 3160.7 |
| $4JKM_1$ | 89.2 | 84.6 | 20.5 | 8.6 | 5.2 | 15.4 | 50.3 | 3328.0 |
| $8JKM_1$ | 89.3 | 84.7 | 20.7 | 7.4 | 5.5 | 15.4 | 51.1 | 3243.1 |
| $0JKM_2$ | 90.0 | 88.8 | 20.3 | 12.3 | 6.6 | 11.2 | 49.6 | 3577.8 |
| $8JKM_2$ | 89.9 | 88.7 | 20.7 | 10.4 | 5.8 | 11.3 | 51.8 | 3540.9 |

JK = *J. curcas* Kernel, JKM = *Jatropha curcas* Kernel Meal, $0JKM_{1-2}$ = control diets,

$4JKM_1$ and $8JKM_{1-2}$ = diets with 4% and 8% jatropha kernel meal in control diet,

DM = Dry Matter, MO = Organic Matter, CP = Crude Protein, EE = Ether Extract, CF = Crude Fiber,

NFE = Nitrogen Free Extract, ME = Metabolic Energy.

**Table 4**: Growth performance characteristics of broiler chickens and chicks during the experiments

| | **0JKM** | **4JKM** | **8JKM** | **P>F** | **SEM** |
|---|---|---|---|---|---|
| Initial weight (g) | 1153.5 | 1132.1 | 1200.6 | 0.5 | 42.0 |
| Final weight (g) | 2078.1[a] | 1407.5[b] | 1089.6[c] | 0.000 | 55.4 |
| $ADFI_1$ (g/d/animal) | 139.2[a] | 55.2[b] | 23.4[c] | 0.000 | 5.8 |
| $ADWG_1$ (g/d/animal) | 61.9[a] | 18.5[b] | -7.7[b] | 0.001 | 6.6 |
| $FCR_1$ | 2.3 | 17.4 | -0.5 | 0.3 | 8.3 |
| Initial weight (g) | 45.7 | - | 46.1 | 0.7 | 0.8 |
| Final weight (g) | 135.5[a] | - | 70.9[b] | 0.000 | 7.4 |
| $ADFI_2$ (g/d/animal) | 18.7[a] | - | 3.1[b] | 0.000 | 1.1 |
| $ADWG_2$ (g/d/animal) | 7.1 | - | 1.9 | 0.17 | 1.2 |
| $FCR_2$ | 3.1 | - | 1.9 | 0.48 | 1.0 |

SEM = Standard Error of the Mean.

[a, b, c] = means with different superscripts on the same row differ significantly ($P<0.05$).

In both experiments ($_1$ and $_2$), ADFI = Average Daily Feed Intake, ADWG = Average Daily Weight Gain, FCR = Feed Conversion Ratio.

## CHAPITRE IV

**Effets de l'incorporation de l'amande de *Jatropha curcas* ayant subi un déshuilage chimique, une fermentation et un traitement thermique dans l'alimentation de poussins de chair en croissance**

**Effects of partial dietary substitution of groundnut meal by defatted, *Aspergillus niger*-fermented and heated *Jatropha curcas* kernel meal on feed intake and growth performance of broiler chicks**

**Article 5 – en rédaction**

Thierry Daniel Tamsir NESSEIM, Moncef BENTEBOULA, Abdoulaye DIENG, Guy MERGEAI, Françoise Marechal & Jean-Luc HORNICK

**Abstract**

This study was conducted to determine intake and growth performance of broiler chicks fed with *Jatropha curcas* kernel meal physico-chemically and biologically processed. The feed experiment lasted for seven days with twenty day-old Ross 308 strain unsexed broiler chicks. Two dietary treatments were given each to ten animals, according to a complete randomized design. Kernels, manually obtained from *J. curcas* seed, were defatted, heated, fermented with a strain of *Aspergillus niger* and oven dried, in order to obtain the treated jatropha kernel meal. This latter was used to replace one-third of a groundnut meal premix which was then incorporated in a commercial diet to warrant iso-nitrogenous and iso-caloric characteristics of the diets. Data collected were analyzed according to ANOVA procedure. The results revealed that animals that received the diet incorporating jatropha kernel meal had numerically higher live weight (156.1 vs. 152.7g/animal) ($P>0.05$) and average daily weight gain (12.3 vs. 11.7g/day/animal) ($P>0.05$) than the control ones, at the end of experiment. The average daily feed intake was the same for the two groups of animals (23.2 g/day/animal) ($P>0.05$) with a similar feed conversion ratio (2.0 vs. 2.1 respectively for the jatropha group and the control group). The survival rate, at the end of the experiment, was 100% for the two groups of animals.

**Keywords:** broiler chicks, *Jatropha curcas*, animal performance, detoxification

## 1. Introduction

*Jatropha curcas* L. belongs to the *Euphorbiaceae* family. It is distributed all over the tropics and subtropics, is not demanding in organic matter, and can grow on degraded soils (Heller, 1996). Fruit produces a seed which contains oil, 25 to 47% of which is extractable (Üllenberg, 2007) by various

methods (Beerens, 2007), and can be used as fuel directly or as substitute to diesel after transesterification (Lu *et al*., 2009).

The meal obtained after oil extraction contains approximately 60% crude protein (Devappa & Swamylingappa, 2008) and is an excellent source of nutrients but the presence of antinutrients and toxic components (Makkar *et al*., 2008). If most of genotypes are toxic (Martinez-Herrera *et al*., 2010), seeds of the non-toxic genotypes, after roasting, can be consumed by humans (Makkar *et. al*., 1998b).

The toxicity of the seed from *J. curcas* was suggested to be due to curcin (Asseleih *et al*., 1989) but more investigations demonstrated that phorbol esters were the most important toxic molecules (Becker & Makkar, 1998; Makkar *et al*., 1997; Makkar *et al*., 1998a; Roach *et al*., 2012). These are diterpenoid esters tigliane polyunsaturated represented by the 12-deoxy-16 hydroxyphorbol (Haas *et al*., 2002) which activate protein kinase C resulting in cytotoxicity (Oskoueian *et al*., 2011). They exhibit also, insecticidal and molluscicidal activities over a wide range of organisms (Wink *et al*., 1997). The oil extraction from the seed has an influence on the level of phorbol esters (Beerens, 2007) which are most concentrated in the kernel of the seed (He *et al*., 2011). Outside phorbol esters, jatropha meal contain, non-only curcin which is capable inhibit protein synthesis (Lin *et al*., 2003), but also antinutrients included trypsin inhibitor, phytate and saponins (Francis *et al*., 2002). Curcin and trypsine inhibitor could interfere with physiological process of monogastrics causing severe growth depression (Palacios *et al*., 2004) but they may be removed by heat and biological treatment (Aderibigbe *et al*., 1997; Aregheore *et al*., 1998; Abou-Arab & Abou-Salem, 2010; Sumiati *et al*., 2012). A reduction of more than half the content of tannins, saponins and phytates, which are heat resistant, was additionally obtained after a fermentation of jatropha meal with different combination of fungi (Belewu *et al*., 2011a; Oseni & Akindahunsi, 2011).

Efforts are under way to detoxify jatropha seed by removing phorbol esters or develop varieties that are deprived of this molecule so that the meal could be used as an ingredient in livestock diet (Makkar & Becker, 2009) and without risk to human health associated with phorbol esters (King *et al*., 2009). On the other hand, the toxic genotype could be utilized as a fertilizer (Nithiyanantham *et al*., 2012). Jatropha kernel meal from the non-toxic genotype is an excellent fish feed (Makkar *et al*., 1999).

Many processing methods have been explored to detoxify meal of *J. curcas* with different levels of success. These include physical (Aregheore *et al*., 1998) and chemical (Haas & Mittelbach, 2000; Aregheore *et al*., 2003) methods; the combination of these two (Martinez-Herrera *et al*., 2006); and biological methods (Belewu & Sam, 2010; Joshi *et al*., 2011). But chemical de-oiling of jatropha kernel, followed by a physico-chemical treatment did not cause a complete removal of phorbol esters (Kumar *et al*., 2010).

Senegal is strongly concerned by the threat of increased imports, especially poultry. This is due to the fact that the country belongs to the category of "least developed country" with a gross national product relatively low (Adjamagbo & Antoine, 2002) and an economy that hardly manages to answer the explosion of urban growth. Despite this, the poultry sector which includes rural and industrial poultry has attracted many investors and is well structured to deal with a segmented market and a variable demand depending on the period (Duteurtre *et al*., 2005). Development of this sector requires a mastery of raw materials used in feed, especially the import of soybean meal which worldwide demand remains strong (Bertrand & Thery, 2006).

The objective of this study was to evaluate, the impact of groundnut cake partial substitution with *J. curcas* kernel meal from Senegal, which was subjected to combined chemical, biological and thermal treatments in order to remove phorbol esters and anti-nutritionals compounds out of the product in a diet, on ingestion and growth performance of broiler chicks.

## 2. Materials and methods

### 2.1. Location of the experiment

Experiment was conducted in *Ecole Nationale Supérieure d'Agriculture* (*ENSA*), University of Thies (Senegal) just after the rainy season (November) with a temperature ranging from 25.7 to 35.1°C and a relative humidity ranging from 36.0 to 39.8%.

### 2.2. Collection and processing of jatropha seeds

Five hundred grams of mature and dry seeds of *Jatropha curcas* were collected from Dialacoto, Senegal. The seeds were weighed and cracked individually to remove the kernel. The kernel was later milled using grinder and then defatted in a Soxhlet type extractor using diethyl ether (boiling point, 60-80°C) several times until the evaporation of residual ether, resulting in meal (defatted kernel).

### 2.3. Source, culture of *Aspergillus niger* and inoculation procedure

Three strains of *Aspergillus niger* van Thieghem were obtained from Belgian Coordinated Collections of *Microorganismes/Mycothèque de l'Université Catholique de Louvain* (Agro) Industrial Fungi and Yeast Collection, Louvain-la-Neuve, Belgium (BCCM™/MUCL 19001, 29039 and 19002) and maintained on Potato-Dextrose-Agar (PDA) medium. Ten ml of spore suspension concentrated to $1.10^6$ in water in 0.05% Tween 80 were used as inoculum.

The substrate, consisting of jatropha kernel meal, was spread into a dish , moistened with distilled water in the ratio of 1:1.5, w/v (62% initial moisture content) and autoclaved at 121°C for 30mn so as to get rid of any microbes that could be present in the meal. Petri dish was inoculated with spore suspension ($1.10^6$spores/ml); the content were mixed and incubated at 39°C for ten days. The fermented substrate was oven dried at 70°C for 48 hours to terminate the fungi growth. The spent substrate, Fermented Jatropha Kernel Meal (FJKM) was later used in the formulation of diet.

### 2.4. Diets preparation

Two broiler starter diets were formulated. The control diet (0FJKM) contained 2/3 of a complete starter commercial feed (SEDIMA S.A.) for broiler chicks and 1/3 of a mixture of groundnut meal (160g), corn (480g), disodium phosphate (32g), and calcium carbonate (32g). The experimental diet (8FJKM) was formulated to contain 2/3 of commercial feed and 1/3 of the previous mixture but whose groundnut meal was replaced with FJKM (**Table 1**).

These rations were iso-nutrients (N.R.C., 1977).

### 2.5. Experimental animals and management

Twenty unsexed one-day old broiler chicks Ross 308 strain were used for the study. The birds were divided into two groups of ten chicks (control group -CG- and jatropha group -JG-) assigned to any of the two diets in a completely randomized design. Animals were kept in a well-ventilated broiler chickens barn in which two areas, separated by fences of 0.75m in height and each area was 2.25m².

During the test, animals were heated by electric light ensuring thermal comfort and water was available *ad libitum*. Feed was weighted early in the morning and provided once a day. Refusals of feed were collected and weighed the day after the distribution.

The study was carried out for seven days.

### 2.6. Data collection

Data were collected on feed intake, weight gain, feed conversion ratio and mortality.

The daily feed intake was estimated as the difference between the feed supplied and the feed rejected over 24 hours period. The Average Daily Feed Intake (ADFI) was then estimated.

Birds in each replicate were individually weighed at the beginning and the end of experiment. The Average Daily Weight Gain (ADWG) was estimated.

The Feed Conversion Ratio (FCR) was determined as the feed intake per unit weight gain.

Mortality was recorded in each replicate and expressed as a percentage of the total number of birds in the replicate at the beginning of the experiment.

### 2.7. Chemical analyses

Samples of jatropha kernel meal, fermented jatropha kernel meal, control diet and diet incorporating the fermented jatropha kernel meal were analyzed for Dry Matter (DM), Crude Protein (CP), Crude Fiber (CF), Ether Extract (EE) and ash using the methods of AOAC (1990) while nitrogen free extract was determined by difference.

The following values were calculated from those measured:

Organic matter (OM) = 100 – Ash

Non-Nitrogen Extract (NNE) = OM – EE – CP – CF

ME = metabolic energy (kcal/kg DM) = 3951 + (54.4 x EE) – (88.7 x CF) – (40.8 x Ash) (Sibbald, 1976)

### 2.8. Statistical analysis

All data generated were subjected to analysis of Variance for Complete Randomized Design with Statistix 8.1 software package. Significant means were separated using Tukey HSD all-pairwise comparisons test of the same package.

## 3. Results

### 3.1. Chemical composition of feed

**Table 2** shows the proximate composition of the experimental diets. Both 0FJKM and 8FJKM showed almost similar value, with regard to the DM, CP, EE, ash and CF. The true metabolic energy of each

diet was 3551 and 3328kcal/kg DM for respectively 0FJKM and 8FJKM. Analytical results showed that diets were almost iso-proteic and iso-energetic.

Concerning the jatropha kernel meal, the treatment with *Aspergillus niger* resulted in a decrease of organic matter, crude protein, ether extract and crude fiber, respectively from 901, 618, 50, 82g/kg DM to 874, 598, 40 and 40g/kg DM.

### 3.2. Feed intake

**Table 3** shows the daily individual feed intake of broiler chicks during the experimental sequence. No significant differences were observed in the feed intake of the two groups of animals (23.2±6.1g/d/animal in CG vs. 23.2±5.5g/d/animal in JG).

Daily feed intake evolution of the different groups is shown in **Figure 1**. For both groups of animals, a similar feed intake was noted, with a decrease on the sixth day, which was related to an insufficient amount of diet.

### 3.3. Growth performance

**Table 3** shows synthetic body weights changes over the experiment. During the seven days, it was found that the control group showed a linear weight growth, evolving from 74.4±8.7g on day 1 (d1) to 152.7±26.3g on day 7 (d7). For the same period, animals that received the fermented jatropha kernel meal diet had the same profile, from 76.2±6.2g to 156.0±9.7g (**Figure 2**). Thus, ADWG per animal did not change significantly for both groups regardless the rate of fermented jatropha kernel meal incorporation, from 11.7g/d/animal for CG to 12.3g/d/animal for JG.

During experiment, no mortality was recorded in any group.

The FCR presented the same mean values, 2.1 and 2.0, respectively for CG and JG without significant difference ($P>0.05$).

## 4. Discussion

Oil extraction from *Jatropha curcas* seeds can be done according to mechanical or chemical processes. Mechanical extraction by means of a screw press is the method used generally in developing countries because of the simplicity of the equipment required (Eckart & Henshaw, 2012). Depending on the level of adjustment of certain parameters (Pradhan *et al.*, 2011), it was possible to recover more than 80% of the oil (Tambunan *et al.*, 2012). However, solvent extraction could be regarded as the most

ideal extraction method since it could recover 95-98% mass fraction of the available oil in the seed (Gübitz *et al.*, 1999).

In our study, because of the non-digestibility of hulls' fibers for monogastrics (Jørgensen *et al.*, 1996), the jatropha seeds were manually shelled before being processed. The kernel obtained was crushed and completely de-oiled by the soxhlet method. By de-oiling, the aim was to obtain a significant reduction of toxic compounds, allowing animals to ingest the jatropha meal. But previous studies (Makkar *et al.*, 1998a; Martinez-Herrera *et al.*, 2006) have shown that de-oiling did not allow this significant reduction. An additional biological fermentation with *Aspergillus niger* was then considered in the light of previous observations that were made (Belewu & Akande, 2010; Belewu & Sam, 2010; Rosa *et al.*, 2010; Brand *et al.*, 2000). To complete the fermentation process, jatropha meal was treated by passage in an autoclave (120°C for 30mn) before inoculation. The fermented substrates were thereafter oven dried at 70°C for 48 hours to terminate the fungi growth and dry the cake. The aim was to inactivate toxic and anti-nutritional compounds. Martinez-Herrera *et al.* (2006), by heat treatment in an autoclave (121°C for 20mn), significantly inactivated trypsin inhibitor activities which are anti-nutritional factors but essentially lectin activity which is considered to be another toxic factor in *J. curcas* seeds. In the same way, Abo El-Fadel *et al.* (2011) decreased the concentration of trypsin inhibitor and lectin by about 75 and 83% respectively. These results were in agreement with Haas & Mittelbach (2000) and Makkar *et al.* (2008) who reported also that heat treatment has a positive effect on reducing trypsine inhibitor and lectine concentration in *J. curcas* meal.

Despite of the FJKM incorporation, the daily feed intake per broiler chicks that received this diet did not vary significantly compared to those who received the control diet. Our results confirmed those of Sumiati *et al.* (2009) who observed that fermented *J. curcas* did not influence feed consumption and body weight gain. These results were contrary to the observations of those obtained by Sumiati *et al.* (2012) which incorporated fermented *J. curcas* meal using *Rhizopus oligosporus* at 7.5% in the diet of laying hens. They obtained a significant decrease in feed consumption and increase of feed conversion ratio.

Jatropha kernels meal biologically treated are reported to remain toxin, even in ruminants. Belewu & Akande (2010) submitted goats to diets in which jatropha meal, fermented with *Penicellium sp* or

*Aspergillus niger*, was partially or fully incorporated to replace soybean meal. Although dry matter intake was lower than for the control group tested, animals showed overall a good ingestion without effects on blood parameters. Similar observations were made by Belewu *et al.* (2010c) with goats receiving diets partially incorporated with jatropha meal fermented with *Rhizospus oligosporus*. These authors showed that despite the low feed intake in tested group, animals showed higher crude protein intake probably due to the higher crude protein content of the diet. Belewu *et al.* (2011b) and Belewu *et al.* (2010a) had yet, with goats also, obtained better feed and nutrient intake and significant higher weight gain with diets that have incorporated fermented jatropha meal successively with *Aspergillus niger*, *Penicillium chrysogenum* and *Trichoderma harzanium.*

By cons, jatropha meal fermented with respectively, *A. niger* and *Trichoderma longibrachiatum*, caused persistent diarrhea, poor feed intake, dehydration and death in goats when it fully substituted to soybean meal in diet (Belewu *et al.*, 2010b).

Despite improved feed intake obtained with diets in which 4 and 6% of jatropha kernel meal fermented with *A. niger* were incorporated, birds did not have a better weight gain and presented, also, smaller internal organs (Oladunjoye *et al.*, 2014). Authors hypothesized that poor feed conversion could be attributed to residues of anti-nutritional and toxic factors. Even at very low levels, phorbol esters may negatively interfere on feed intake (Sumiati *et al.*, 2012). Ojediran *et al.* (2014) observed also a growth depression due to residual anti-nutritional factors.

Because of the presence of phorbol esters, jatropha meal presented, for animals, a very bad palatability. Indeed, Aregheore *et al.* (2003) reported that a concentration of 0.13mg/g phorbol esters present in the jatropha meal has a significant adverse effect on feed intake. Moreover, protein isolates produced by papain treatment and associated with *Panicum maximum*, induced a low ingestion in guinea pigs (Kouakou *et al.*, 2010).

Veerabhadrappa *et al.* (2014) have shown that solid-state fermentation of jatropha seed cake using *Aspergillus versicolor* reduced by about 76% phorbol esters and significantly anti-nutrients like phytic acid, tannins, trypsin inhibitors, cyanogenic glucosides and lectins. Made under optimum conditions, fermentation by *Pseudomonas aeruginosa* PseA, carried out on a substrate consisting of a jatropha seed meal, was allowed to completely degrade phorbol esters in nine days (Joshi *et al.*, 2011).

Monogastrics generally exhibit a high sensivity to the presence of phorbol esters and other anti-nutritional factors (Becker & Makkar, 1998; Rakshit *et al.*, 2008) but, in our study, their feed behavior was not significantly affected by incorporation of treated jatropha kernel meal in the diet, on the contrary. In addition, the possible presence of toxic factors did not affect the viability of the animals. This was confirmed by previous studies (Annongu *et al.*, 2010; Belewu & Akande, 2010; Oladunjoye *et al.*, 2014). They confirmed that the residual toxic and anti-nutritional components in the jatropha kernel meal biologically treated did not reach the lethal dose for animals.

Fungi could be considered the most suitable organisms to fermentation solid substrate because their hyphae could colonize the substrate by penetrating the interparticle spaces (Pandey *et al.*, 1999). The application of this technology has enabled the development of degradation of hazardous compounds, biological detoxification of crop residues for enrichment of nutrition and production of value added products (Dos Santos *et al.*, 2004). Biological processes through use of fungi, bacteria or enzyme complexes, could allow a significant reduction of toxic and anti-nutritional compounds and in some cases, improve the nutritional value of meal and therefore its use in animal feed (Nesseim *et al.*, 2014). The nitrogen level of jatropha kernel meal increased after treatment with *Aspergillus niger*. This is probably due to the fermentation of non-nitrogen compounds, some volatile end products escaping from the media, thus leaving higher relative amounts of nitrogen. Seed cake and fruit pulp then can be used for biogas production by fermentation (Vyas & Singh, 2007).

In our case, the kernel of jatropha was de-oiled, fermented and heat treated. These treatments have probably resulted in a significant decrease of phorbol esters but also in most of anti-nutritional compounds and improved the feed consumption and weight gain, contrary to what had been observed in our previous study (Nesseim *et al.*, 2015). To our knowledge, such positive results are reported for the first time in the literature, and anyway in broiler chick production. This suggests that biological and physical treatments presumably allowed the remove of phorbol esters, curcin and some anti-nutritionnal factors such as trypsine inhibitors. Considering the reaction of the animals after the intake of tested feed, it can be conclude that a large quantity of toxic compound has probably been eliminated in the jatropha kernel meal. Fermentation of Jatropha kernel meal with *A. niger* followed by heat

treatment was probably a adequate method for suppressing phorbol esters. Moreover, the treatment has even positively affected feed intake and weight and it had no impact on the viability of animals.

This study was the first field experiment on evaluation of jatropha kernel seed fermented with *Aspergillus Niger* in broiler chicks feeding in Senegal. The results showed that, after a total dehulling, a chemical de-oiling with diethyl ether as well as a biological and heat treatment, jatropha kernel stop impacting feed intake and growth of chicks. Further studies must be performed in order to confirm the use of fungal fermentation to allow detoxification of *Jatropha curcas* meal.

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**References**

1. ABOU-ARAB A.A. & ABOU-SALEM M.F., 2010. Nutritional quality of *Jatropha curcas* seeds and effect of some physical and chemical treatments on their nutritional factors. African Journal of Food Science, **4**(3), 93-103.
2. ABO El-FADEL M.H., HUSSEIN A.M. & MOHAMED A.H., 2011. Incorporation *Jatropha curcas* meal on lambs ration and it's effect on lambs performance. Journal of American Science, **7**(2), 129-132.
3. ADERIBIGBE A.O., JOHNSON C.O.L.E., MAKKAR H.P.S., BECKER K. & FOIDL N., 1997. Chemical composition and effect of heat on organic matter- and nitrogen-degradability and some antinutritional components of jatropha meal. Animal Feed Science and Technology, **67**(2), 223-243.
4. ADJAMAGBO A. & ANTOINE P., 2002. Le Sénégal face au défi démographique. Document de travail DIAL nDT/2002/07, 28p.
5. ANNONGU A.A., JOSEPH J.K., APATA D.F., ADEYINA A.O., YOUSUF M.B. & OGUNJIMI K.B., 2010. Detoxification of *Jatropha curcas* seeds for use in nutrition of monogastric livestock as alternative feedstuff. Pakistan Journal of Nutrition, **9**(9), 902-904.

6. AOAC, 1990. Official Methods of Analysis (Volume 1). 15th Edn. Association of Official Analytic Chemists, Washington DC., USA.
7. AREGHEORE E.M., MAKKAR H.P.S. & BECKER K., 1998. Assessment of lectin activity in a toxic and a non-toxic variety of *Jatropha curcas* using latex agglutination and haemagglutination methods and inactivation of lectin by heat treatments. Journal of the Science of Food and Agriculture, **77**(3), 349-352.
8. AREGHEORE E.M., BECKER K. & MAKKAR H.P.S., 2003. Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats. The South Pacific Journal of Natural Science, **21**(1), 51-56.
9. ASSELEIH L.M.C., PLUMBLEY R.A. & HYLANDS P.J., 1989. Purification and partial characterization of a hemagglutinin from seeds of *Jatropha curcas*. Journal of Food Biochemistry, **13**(1), 1-20.
10. BECKER K. & MAKKAR H.P.S, 1998. Effects of phorbol esters in carp (*Cyprinus carpio* L.). Veterinary & Human Toxicology, **40**(2), 82-86.
11. BEERENS P., 2007. Screw-pressing of jatropha seeds for fuelling purposes in less developed countries. Msc Dissertation, Department of Sustainable Energy Technology, Eindhoven University of Technology, Eindhoven. 80p.
12. BELEWU M.A., AHMED O. & IBRAHIM S.O., 2011a. Solid state fermentation of *Jatropha curcas* with cocktail of fungi. International Journal of Biosciences, **1**(1), 12-19.
13. BELEWU M.A. & AKANDE B.A., 2010. Biological upgrading of the nutritional quality of *Jatropha curcas* kernel cake: effect on performance characteristics of goat. International Research Journal of Biotechnology, **1**(2), 19-22.
14. BELEWU M.A., BELEWU K.Y. & LAWAL I.A., 2011b. Cocktail of fungi blend on *Jatropha curcas* kernel cake: effect on feed intake and blood parameters of goat. Lybian Agriculture Research Center Journal International, **2**(3), 138-143.
15. BELEWU M.A., BELEWU K.Y. & POPOOLA L.A., 2010a. Effect of cocktail of fungi blend on the digestibility coefficient and digestible nutrients of goat (*Capra hircus*). British Biotechnology Journal, **1**(2), 46-52.

16. BELEWU M.A., BELEWU K.Y. & OGUNSOLA F.O., 2010b. Nutritive value of dietary fungi treated *Jatropha curcas* kernel cake: voluntary, growth and digestibility coefficient of goat. Agriculture and Biology Journal of North America, **1**(2), 135-138.
17. BELEWU M.A., ENIOLORUNDA O.O. & LLORI G., 2010c. Response of goat to fungi (*Rhizopus Oligosporus*, *Rhizopus nigrican*) treated *Jatropha curcas* kernel cake. Archives of Applied Science Research, **2**(4), 255-261.
18. BERTRAND J.P. & THERY H., 2006. Le marché mondial et l'expansion du « complexe soja » dans les cerrados du Mato Grosso. La mondialisation côté Sud. Acteurs et territoires, IRD, Paris : Editions ENS, 67p.
19. BELEWU M.A. & SAM R., 2010. Solid state fermentation of *Jatropha curcas* kernel cake: proximate composition and antinutritional components. Journal of Yeast and Fungal Research, **1**(3), 44-46.
20. BRAND D., PANDEY A., ROUSSOS S. & SOCCOL C.R., 2000. Biological detoxification of coffee husk by filamentous fungi using a solid state fermentation system. Enzyme and Microbial Technology, **27**(1-2), 127-133.
21. DEVAPPA R.K. & SWAMYLINGAPPA, 2008. Biochemical and nutritional evaluation of jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors. Journal of the Science of Food and Agriculture, **88**(5), 911-919.
22. DUTEURTRE G., DIEYE P.N. & DIA D., 2005. L'impact des importations de volailles et de produits laitiers sur la production locale au Sénégal. Etudes et documents « Ouverture des frontières et développement agricole dans les pays de l'UEMOA », ISRA-BAME, **8**(1), 78p.
23. Dos SANTOS M.M., Da ROSA A.S., DAL'BOIT S., MITCHELL D.A. & KRIEGER N., 2004. Thermal denaturation: is solid-state fermentation really a good technology for the production of enzymes? Bioresource Technology, **93**(3), 216-268.
24. ECKART K. & HENSHAW P., 2012. *Jatropha curcas* L. and multifunctional platforms for the development of rural sub-Saharan Africa. Energy for Sustainable Development, **16**(3), 303-311.
25. FRANCIS G., MAKKAR H.P.S. & BECKER K., 2002. Products from little researched plants as aquaculture feed ingredients. AGRIPPA (FAO) peer-reviewed electronic journal.

26. GÜBITZ G.M., MITTLEBACH M. & TRABI M., 1999. Exploitation of the tropical oil seed plant Jatropha curcas L. Bioresource Technology, **67**(1), 73-82.

27. HAAS W. & MITTLEBACH M., 2000. Detoxification experiments with the seed oil from *Jatropha curcas* L. Industrial Crops and Products, **12**(2), 111-118.

28. HAAS W., STERK H. & MITTLEBACH M., 2002. Novel 12-deoxy-16-hydroxyphorbol diesters isolated from the seed oil of *Jatropha curcas*. Journal of Natural Products, **65**(10), 1434-1440.

29. HE W., KING A.J., KHAN M.A., CUEVAS J.A., RAMIARAMANANA D. & GRAHAM I.A., 2011. Analysis of seed phorbol-ester and curcin content together with genetic diversity in multiple provenances of *Jatropha curcas* L. from Madagascar and Mexico. Plant Physiology and Biochemistry, **49**(10), 1183-1190.

30. HELLER J., 1996. Physic nut. *Jatropha curcas* L. promoting the conservation and use of underutilized and neglected crops. Institute of Plant Genetics and Crop Plant Research Notes, Gatersleben / International Plant Genetic Resources Institute, Rome Italy, 66p.

31. JØRGENSEN H., ZHAO X.-Q., KNUDSEN K.E.B. & EGGUM B.O., 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. British Journal of Nutrition, **75**(3), 379-395.

32. JOSHI C., MATHUR P. & KHARE S.K., 2011. Degradation of phorbol esters by *Pseudomonas aeruginosa* PseA during solid-state fermentation of deoiled *Jatropha curcas* seed cake. Bioresource Technology, **102**(7), 4815-4819.

33. KING A.J., HE W., CUEVAS J.A., FREUDENBERGER M., RAMIARAMANANA D. & GRAHAM A., 2009. Potential of *Jatropha curcas* as a source of renewable oil and animal feed. Journal of experimental Botany, **60**(10), 2897-2905.

34. KOUAKOU N.D.V., THYS E., ASSIDJO E.N. & GRONGNET J.F., 2010. Ingestion et digestibilité *in vivo* du *Panicum maximum* associé à trois compléments: tourteau de *Jatropha curcas*, tourteau de coton (*Gossipium hirsutum*) et *Euphorbia heterophylla* chez le cobaye (*Cavia porcellus* L.). Tropicultura, **28**(3), 173-177.

35. KUMAR V., MAKKAR H.P.S. & BECKER K., 2010. Nutritional, physiological and haematological responses in rainbow trout (*Oncorhynchus myskiss*) juveniles fed detoxified *Jatropha curcas* kernel meal. Aquaculture Nutrition, **17**(4), 451-467.

36. LIN J., FANG Y., LIN T. & FANG C., 2003. Antitumor effects of curcin from seeds of *Jatropha curcas*. Acta Pharmacologica Sinica, **24**(3), 241-246.

37. LU H., LIU Y., ZHOU H., YANG Y., CHEN M. & LIANG B., 2009. Production of biodiesel from *Jatropha curcas* L. oil. Computers & Chemical Engineering, **33**(5), 1091-1096.

38. MAKKAR H.P.S., ADERIBIGBE A.O. & BECKER K., 1998a. Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic effects. Food Chemistry, **62**(2), 207-215.

39. MAKKAR H.P.S. & BECKER K., 2009. *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. European Journal of Lipid Science and Technology, **111**(8), 773-787.

40. MAKKAR H.P.S., BECKER K., NITIS I.M. & SHIN M.T., 1999. Plant toxins and detoxification methods to improve feed quality of tropical seeds. Asian-Australasian Journal of Animal Sciences, **12**(3), 467-480.

41. MAKKAR H.P.S., BECKER K. & SCHMOOK B., 1998b. Edible provenances of *Jatropha curcas* from Quintana Roo state of Mexico and effect of roasting on antinutrient and toxic factors in seeds. Plant Foods for human nutrition, **52**(1), 31-36.

42. MAKKAR H.P.S., BECKER K., SPORER F. & WINK M., 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. Journal of Agricultural and Food Chemistry, **45**(8), 3152-3157.

43. MAKKAR H.P.S., MARTINEZ-HERRERA J. & BECKER K., 2008. Variations in seed number per fruit, seed physical parameters and contents of oil, protein and phorbol ester in toxic and non-toxic genotypes of *Jatropha curcas*. Journal of Plant Sciences, **3**(4), 260-265.

44. MARTINEZ-HERRERA J., AYALA A.L.M., MAKKAR H.P.S., FRANCIS G. & BECKER K., 2010. Agroclimatic conditions, chemical and nutritional characterization of different provenances of *Jatropha curcas* L. from Mexico. European Journal of Scientific Research, **39**(3), 396-407.

45. MARTINEZ-HERRERA J., SIDDHURAJU P., FRANCIS G., DAVILA-ORTIZ G. & BECKER K., 2006. Chemical composition, toxic/antimetabolic constituents, and effects if different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. Food Chemistry, **96**(1), 80-89.

46. NITHIYANANTHAM S., SIDDHURAJU P. & FRANCIS G., 2012. Potential of *Jatropha curcas* as a biofuel, animal feed and products. Journal of the American Oil Chemists' Society, **89**(6), 961-972.

47. NESSEIM T.D.T., DIENG A., MERGEAI G. & HORNICK J.-L., 2014. Toxicité et détoxification biologique du tourteau de *Jatropha curcas* L. pour une utilisation en alimentation animale : synthèse bibliographique. Revue Africaine de Santé et de Productions Animales, **12**(3-4), 143-149.

48. NESSEIM T.D.T., DIENG A., MERGEAI G., NDIAYE S. & HORNICK J.-L., 2015. Digestibility of solvent-treated *Jatropha curcas* kernel by broiler chickens in Senegal. Tropical Animal Health and Production, **47**(8), 1553-1560.

49. N.R.C., 1977. Nutrient requirements of domestic animals, Number 1, Nutrient requirements of poultry. Seventh revised edition, National Academy of Sciences. Washington, D.C., 61p.

50. OLADUNJOYE I.O., OJEDIRAN T., ARINGBANGBA C., AKINRINLADE O.S. & OPAKUNLE O.G., 2014. Effects of inclusion level and length of fermentation on the utilization of jatropha (*Jatropha curcas*) seed cake by broiler chickens. International Journal of Current Microbiology and Applied Sciences, **3**(7), 44-54.

51. OJEDIRAN T.K., ADISA Y.A., YUSUF S.A. & EMIOLA I.A., 2014. Nutritional evaluation of processed *Jatropha curcas* kernel meals: effects on growth performance of broiler chicks. Journal of Animal Science Advances, **1**(11), 1110-1121.

52. OSENI O.A. & AKINDAHUNSI A.A., 2011. Some phytochemical properties and effect of fermentation on the seed of *Jatropha curcas* L. American Journal of Food Technology, **6**(2), 158-165.

53. OSKOUEIAN E., ABDULLAH N., AHMAD S., SAAD W.Z., OMAR A.R. & HO Y.W., 2011. Bioactive compounds and biological activities of *Jatropha curcas* L. kernel meal extract. International Journal of Molecular Sciences, **12**(9), 5955-5970.

54. PALACIOS M.F., EASTER R.A., SOLTWEDE K.T., PARSONS C.M., DOUGLAS M.W., HYMOWITZ T. & PETTIGREW J.E., 2004. Effect of soybean variety and processing on growth performance of young chicks and pigs. Journal of Animal Science, **82**(4), 1108-1114.

55. PANDEY A., SELVAKUMAR P., SOCCOL C.R. & NIGAM P., 1999. Solid state fermentation for the production of industrial enzymes. Current Science, **77**(1), 149-162.

56. PRADHAN R.C., MISHRA S., NAIK S.N., BHATNAGAR N. & VIJAY V.K., 2011. Oil expression from jatropha seeds using a screw press expeller. Biosystems Engineering, **109**(2), 158-166.

57. RAKSHIT K.D., DARUKESHWARA K., RATHINA RAJ K., NARASIMHAMURTHY K., SAIBABA P. & BHAGYA S., 2008. Toxicity studies of detoxified jatropha meal (*Jatropha curcas*) in rats. Food and Chemical Toxicology, **46**(12), 3621-3625.

58. ROACH J.S., DEVAPPA R.K., MAKKAR H.P.S. & BECKER K., 2012. Isolation, stability and bioactivity of *Jatropha curcas* phorbol esters. Fitoterapia, **83**(3), 586-592.

59. ROSA T.D.S., CASTRO A.M., TORRES A.G. & FREIRE D.M., 2010. Analysis of nutritional composition and detoxification of *Jatropha curcas* cake after solid-state fermentation. In the 32nd Symposium on Biotechnology for Fuels and Chemicals, Florida, 12-29.

60. SIBBALD, I.R., 1976. The true metabolizable energy values of several feeding stuffs measured with roosters, laying hens, turkeys and broiler hens. Poultry Science, **55**(4), 1459-1463.

61. SUMIATI S., MUTIA R. & DAMANSYAH A., 2012. Performance of layer hen fed fermented *Jatropha curcas* L. meal supplemented with cellulose and phytase enzyme. Journal of Indonesian Tropical Animal Agriculture, **37**(2), 108-114.

62. SUMIATI Y.Y., ASTUTI D.A. & SUHARTI S., 2009. Feeding fermented *Jatropha curcas* L. meal supplemented with cellulose and phytase to kampong chicken. In: Proceeding, the 1st International Seminar on Animal Industry, Faculty of Animal Science, Bogor Agricultural University, Bogor, 23-24.

63. TAMBUNAN A.H., SITUMORANG J.P., SILIP P.P., JOELIANINGSIH A. & ARAKI T., 2012. Yield and physicochemical properties and mechanically extracted *Jatropha curcas* L. oil. Biomass and Bioenergy, **43**, 12-17.

64. ÜLLENBERG A., 2007. Jatropha à Madagascar -Rapport sur l'état actuel du secteur- Gesellschaft für Technische Zusammenarbeit (GTZ), Madagascar, 32p.

65. VEERABHADRAPPA M.B., SHIVAKUMAR S.B. & DEVAPPA S., 2014. Solid-state fermentation of jatropha seed cake for optimization of lipase, protease and detoxification of anti-nutrients in jatropha seed cake using *Aspergillus versicolor* CJS-98. Journal of Bioscience and Bioengineering, **117**(2), 208-214.

66. VYAS D.K. & SINGH R.N., 2007. Feasibility study of jatropha seed husk as an open core gasifier feedstock. Renewable Energy, **32**(3), 512-517.

67. WINK M., KOSCHMIEDER C., SAUERWEIN M. & SPORER F., 1997. Porbol esters of *J. curcas* - Biological activities and potential applications. G.M. Gübtiz, M. Mittlebach, M. Trabi (Eds.), Biofuels and Industrial Products from *Jatropha curcas*, DBV, Graz, 160-166.

**Table 1:** Composition of diets incorporating *J. curcas* kernel meal

| **Raw materials (%)** | **0FJKM** | **8FJKM** |
|---|---|---|
| Complete starter diet | 68.0 | 68.0 |
| Groundnut meal | 8.0 | - |
| FJKM | - | 8.0 |
| Maize | 20.0 | 20.0 |
| Phosphate disodium | 2.0 | 2.0 |
| Calcium carbonate | 2.0 | 2.0 |
| **Total** | **100.0** | **100.0** |

FJKM = Fermented Jatropha Kernel Meal,

0FJKM = control diet, 8FJKM = diet incorporating 8% of fermented jatropha kernel meal.

**Table 2:** Proximate analytical composition of the diets used during the experimentation

| | **DM (%)** | **Chemical composition (% in DM)** | | | | | | **ME** |
|---|---|---|---|---|---|---|---|---|
| | | **OM** | **CP** | **EE** | **CF** | **Ash** | **NNE** | **(kcal/kg DM)** |
| **JKM** | 91.6 | 90.1 | 61.8 | 5.0 | 8.2 | 9.9 | 15.1 | 3091.7 |
| **FJKM** | 93.5 | 87.4 | 59.8 | 4.0 | 4.0 | 12.6 | 19.6 | 3299.7 |
| **0FJKM** | 91.9 | 89.5 | 25.0 | 5.9 | 3.3 | 10.5 | 55.3 | 3550.9 |
| **8FJKM** | 90.3 | 87.3 | 27.4 | 4.1 | 3.7 | 12.7 | 42.1 | 3327.7 |

JKM = Jatropha Kernel Meal, DM = Dry Matter, OM = Organic Matter, CP = Crude Protein,

EE = Ether Extract, CF = Crude Fiber, NNE = Non-Nitrogen Extract.

0FJKM = control diet, 8FJKM = diet incorporating 8% of fermented jatropha kernel meal.

**Table 3**: Growth performance characteristics of broiler chicks receiving or not 8% deoiled, Aspergillus niger-treated and heated *J. curcas* kernel meal

| | **0FJKM** | **8FJKM** | **P>F** | **±SEM** |
|---|---|---|---|---|
| Initial weight (g) | 74.4 | 76.2 | 0.6 | 2.4 |
| Final weight (g) | 152.7 | 156.1 | 0.7 | 6.3 |
| ADFI (g/d/animal) | 23.2 | 23.2 | 0.9 | 2.2 |
| ADWG (g/d/animal) | 11.7 | 12.3 | 0.8 | 1.7 |
| Feed conversion ratio | 2.1 | 2.0 | 0.6 | 0.1 |

SEM = Standard Error of the Mean.

ADFI = Average Daily Feed Intake, ADWG = Average Daily Weight Gain.

0FJKM = control diet, 8FJKM = diet incorporating 8% of fermented jatropha kernel meal.

**Figure 1:** Evolution of feed intake in control group and in group of animals that received a diet containing 8% jatropha kernel meal de-oiled, heated and *Aspergillus niger*-treated

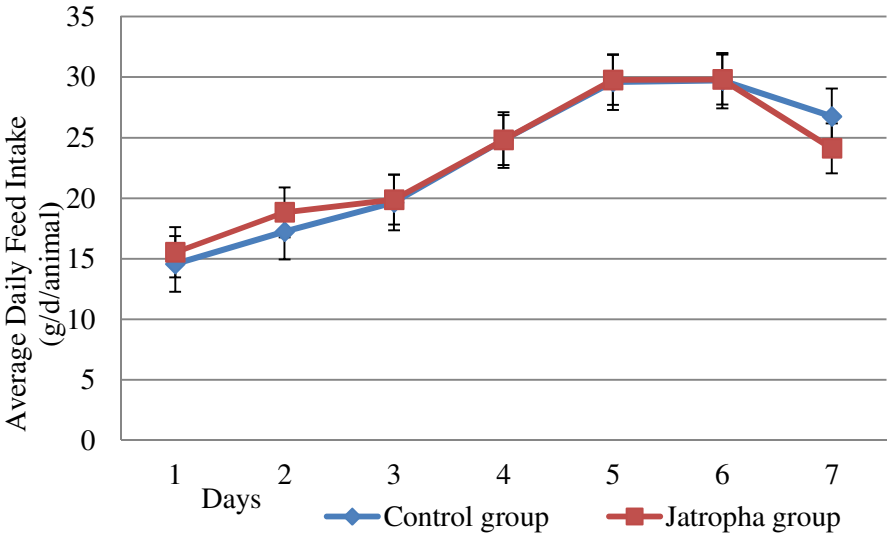
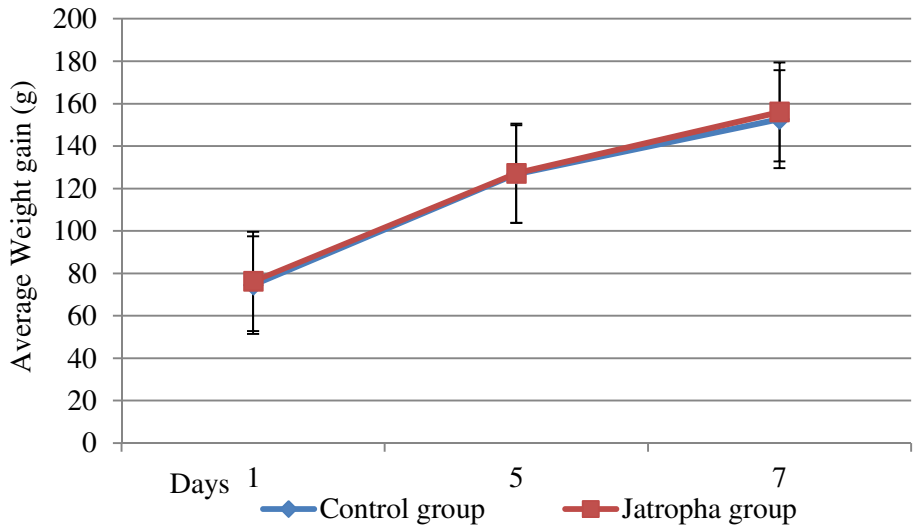



**Figure 2**: Evolution of weight in control group and in group of animals that received a diet containing 8% jatropha kernel meal de-oiled, heated and and *Aspergillus niger*-treated



# CHAPITRE V

## GENERAL DISCUSSION AND PERSPECTIVES

This study made possible to highlight the limits of use of *Jatropha curcas* L. kernel seed in monogastric feeding, particularly in chickens, despite the various treatments applied for detoxification. Most of oilseeds are large sources of vegetal oils but can be also used in animal feed because of their high protein content (McKevith, 2005). Those are mainly meals which are obtained after processing oilseeds and that can contribute to more than half of the total amount of proteins (Dale, 1996) in poultry and livestock diets. The quality of these proteins could be influenced by the degree of transformation (Anderson-Hafermann *et al*., 1993).

In response to a limited supply of protein for animal feed, developing countries have made research efforts to exploit the nutritional potential of some lesser-known oilseeds (Enujiugha *et al*., 2003). These latter often had high levels of protein but with anti-nutritional and toxic factors that protect the plant and cause adverse effects on humans and animals (Makkar *et al*., 1999). Thus, among a number of under-utilized oilseed crops (Eze, 2016), there has been a growing interest in those whose oil is inedible (Balat, 2011). This is the case of *Jatropha curcas* L., whose seeds can be used as an alternative energy and nutritional source (Emil *et al*., 2010; Ugbogu *et al*., 2013) despite their toxicity (Heller, 1996). This one is mainly related to the presence of phorbol esters which are concentrated in the seed kernel (Makkar *et al*., 1998a, Baldini *et al*., 2014). These are polycyclic compounds in which two hydroxyl groups are esterified to fatty acids on neighboring carbon atoms (Goel *et al*., 2007). The most active is 4β-12-*O*-tetradecanoylphorbol-13-acetate (TPA), also known as phorbol 12-myristate-13-acetate (PMA). This compound, which acts at low concentrations (Ghandi *et al*., 1995), possesses inflammatory and tumor-promoting properties. It activates protein kinase C (Oskoueian *et al*., 2012), a phosphorylation enzyme that is stimulated by calcium and phospholipids, present in the central nervous system of vertebrates (Castagna *et al*., 1982). This action results in toxicity, particularly by ingestion, in various animal species (Adam, 1974; Ahmed & Adam, 1979; El Badwi *et al*., 1995; Abdel Gadir *et al*., 2003; Li *et a*l., 2011).

In addition to phorbol esters, curcine also is found in jatropha seed (Aregheore *et al*., 1998). Curcine is a lectin which is close to ricin, classified as a "ribosomal inactivating protein" (Lin *et al*., 2003). It is an antinutritional substance acting on a few internal organs of animals and reducing the activity of digestive enzymes (Meite *et al*., 2006). Also present is the inhibitor of trypsin activity which has got

an antinutritional effect due to its direct interaction with proteolytic enzymes secreted by the pancreas, leading to a reduction in the digestibility of the proteins of the ration (Hajos *et al*., 1995). Finally, phytates are able to form some complexes with proteolytic enzymes, reducing protein digestibility (Ravindran *et al*., 2000) whereas saponins, some triterpenoid glycosides, can affect feed intake owing to their astringent and irritating taste (Francis *et al*., 2002).

Thus, in addition to providing oil, the seed of *J. curcas* produces an oil cake that could be used like a compost (Das *et al*., 2011) rich in nutrients for fertilization (Francis *et al*. 2005), phorbol esters being completely biodegraded in soil by temperature and humidity levels (Devappa *et al*., 2010; Nakao *et al*., 2015). The seed could also be an asset as a cost saving protein supplement but valuable in animal feed if toxic compounds are eliminated.

Different detoxification methodologies have been proposed. These are physical processes (Aregheore *et al*., 1998; Makkar *et al*., 1998b; Siddhuraju *et al*., 2002; Kongmany *et al*., 2014), chemicals processes (Haas *et al*., 2000; Ahmed & Salimon, 2009; Saetae & Suntornsuk, 2010; Phasukarratchai *et al*., 2012; Guedes *et al*., 2014) and biological processes (Belewu & Sam, 2010; Devappa *et al*., 2010; Abo El-Fadel *et al*., 2011; Phengnuam & Suntornsuk, 2013). Taken individually, these detoxification processes have sometimes shown limits. Physical treatments, which inactivate curcine and trypsin inhibitors, do not change the concentration of phorbol esters, or that of phytates and saponins. A reduction in phorbol ester levels is only achieved by chemical and biological treatments, while a combination of the different treatments gives the best results with an impact on toxic and antinutritional compounds (Aregheore *et al*., 2003; Chivandi et al Abu-Salem, 2010; El Diwani *et al*., 2011; Xiao *et al*., 2011).

Despite the lipophilic nature of phorbol esters, studies have shown that protein concentrates obtained from deoiled seeds still exhibit high levels that are likely to cause animal intoxication (Makkar *et al*., 2008; Saetae *et al*., 2011). These phorbol esters appeared to be strongly bound to the protein fragments and were precipitated together with the proteins in the isoelectric precipitation process. This was confirmed by Makkar & Becker (2009) who obtained in that way a substantial amount of phorbol esters and antinutritional factors remaining. Among the latter, trypsin inhibitor and lectins could be

reduced by heat treatments, while adverse effect of phytate could be mitigated by addition of phytase in diets (Makkar, 2016).

It appeared that the production process of these protein isolates plays a fundamental role. Thus, Devappa & Swamylingappa (2008), which extracted proteins at alkaline pH and isoelectric precipitation followed by steam injection and rinsing, reduced the activity of the trypsin inhibitor, phytates and saponins by more than 90%, while the phorbol esters were completely eliminated. In addition, a protein concentrate obtained from previously chemically treated jatropha oil cake, despite its high protein content (89-96%), did not show detectable levels of phorbol esters and curcine (Devappa & Swamylingappa, 2008; Saetae & Suntornsuk, 2011). The same protein concentrate obtained, this time, from a non-toxic variety was free of phorbol esters but contained anti-nutritional factors such as the trypsin inhibitor, lectins and phytates whose level was reduced by heat treatments, producing an excellent supplement for livestock (Makkar & Becker, 2009).

Beerens (2007) and Makkar & Becker (2009) have shown that the extraction of oil method could have an effect on phorbol ester levels, as these molecules are relatively lipophilic (Sharkey & Blumberg, 1985) and will be distributed in the oil obtained and in the remaining meal. The study by Nesseim *et al.* (2015), in view of the quantity of jatropha kernel obtained after manual seed hulling, attempted to combine chemical de-oiling with petroleum ether and pressing to remove residues of oil and petroleum ether. This was the first experiment initiated, in Senegal, from the de-oiling of the jatropha kernel seeds, incorporated into crushed corn at levels of 4, 8 and 12%. The whole, thus constituted, was presented to broiler chickens which were 6 weeks old. The authors attempted to determine the levels of acceptability and ingestion, depending on the incorporation rates but also the overall digestibility of the feed as well as that differential related to the jatropha kernel oil cake. In this way, the authors have attempted to reduce toxic compounds, in particular phorbol esters, in the remaining meal. Although the results obtained from dry matter digestibility were encouraging, the other data showed that during all the experiments the feed intake was inversely proportional to the rate of incorporation of the jatropha. One of the working leads of work has been a notable decrease in the appetite for the feed containing jatropha seed derivatives. These observations were correlated with the amount of fecal matter produced, resulting in decreases of weight in animals that were subjected to diets containing

deoiled jatropha kernel. The digestibility of nutrients particularly that of crude protein, which had been approximately 50% with the control group, decreased significantly with the incorporation of jatropha. It has been deduced that the differential digestibility of crude proteins in jatropha kernel meal has reached negative values for all levels of incorporation, leading to mobilization and excretion of endogenous proteins. Although there was no evidence of acute poisoning or mortality of the poultry, animals showed poor appetite when subjected to diets incorporating jatropha, which resulted in a drop of feed intake and poor weight performance. These results confirmed that despite the low level of ether extract obtained for jatropha kernel oil cake (6.5% on dry matter), the de-oiling treatments only allowed a partial reduction of toxic factors, especially phorbol esters. Similar observations were made by Chivandi *et al.* (2000) in pigs which, in addition to a decrease in daily weight gain, showed an increase in the fragility of the red blood cells.

After de-oiling, most of the phorbol esters are mainly found in oil. Indeed, Ojo *et al.* (2013) supplemented poultry rations with 4, 8 and 12% of non-deoiled jatropha kernel seeds and observed, in addition to increased mortality based on incorporation of jatropha, also decreased growth as well as liver and kidney damage. This was corroborated by the acute toxicity provoked in rats resulting in severe diarrhea associated with inflammation of the digestive tract (Ghandi *et al.*, 1995) and decreased fertility (Odusote *et al.*, 2002). Devappa & Swamylingappa (2008) showed later that a thermal treatment of the oil cake resulting in the elimination of anti-nutritional compounds improved the digestibility of proteins. On the other hand, Chivandi *et al.* (2006) showed that a meal treated with hexane followed by ethanol did not allow complete removal of phorbol esters causing skin lesions, persistent diarrhea and alteration of blood parameters in pigs fed with treated jatropha meal. Katole *et al.* (2011) also showed that the treatment of jatropha meal with sodium chloride in combination with calcium hydroxide did not increase feed intake and apparent digestibility of nutrients in sheep despite decrease phorbol esters content. The same observations were made with heifers that received ethanol-treated jatropha meal (Da Silva, 2015). Rakshit *et al.* (2008) demonstrated a significant reduction in dietary intake and a decrease in weight gain related to the residual amounts of phorbol esters and anti-nutrients in rats. These compounds, without causing mortality, could lead to a decrease in productivity

(Francis *et al.*, 2001). Finally, Sudake *et al.* (2013) observed a negative effect on growth performance in calves that received a mix of concentrate incorporating lemon-treated jatropha oil cake.

Thus, in monogastric animals, better gastric digestibility of meal could be obtained by better use of proteins with the non-toxic variety (Selje-Assmann *et al.*, 2007; Félix-Bernal *et al.*, 2014), which does not present any deleterious effect on the health of animals (Martinez-Herrera *et al.*, 2012).

All these observations were confirmed by Nesseim *et al.* (article accepted in publication). The authors successively evaluated the impact of the incorporation of de-oiled jatropha kernel into a commercial ration for growing broiler chickens and the impact of the incorporation of de-oiled and heated jatropha kernel in a feed ration for chicks in the start-up phase. In these two experiments, one-day and 30-day old broiler chickens were subjected to different diets, starting from the observations made by Nesseim *et al.* (2015). The first diet consisted of incorporating 4 and 8% jatropha oil cake in a diet prepared with a commercial feed re-balanced with corn so as to make it isoproteinic and isoenergetic. The second diet consisted of a prepared ration in which peanut oil cake had been partially substituted by jatropha meal heated in an oven and incorporated into 8% of the total ration. In both cases, the animals subjected to these different diets systematically showed a reduction in their feed intake according to the level of incorporation of the jatropha oil cake resulting in a decrease in the weight gain compared to the different control groups. In addition, mortality rates ranged from 5 to 20% depending on the rate of incorporation of jatropha oil cake in the first experiment, compared with 60% in the second experiment. The anatomo-pathological observations made on the organs of the animals (mouth, esophagus, crop, proventricule, gizzard, intestines, liver, pancreas and kidneys) did not reveal any obvious lesions. We were not equipped to easily make histological cuts examination of these organs. Rakshit *et al.* (2008), despite different treatments on jatropha meal, observed, in addition to a decrease in feed intake and weight loss, successive mortalities in rats but the organs analyzed showed no macroscopic or histological abnormalities. Animal mortality may have been linked to the lack of feed intake and resulting wasting, as noted by Rahma *et al.* (2013). The resulting weight loss, particularly in growing animals, could lead to a decline in immunity, exposing animals to various germs, even non-pathogenic ones. This is particularly likely in selected strains, where tolerated mortality is already in the order of a few percent.

Thus, despite the treatments undergone, the jatropha kernel used in our first experiments has not been found free enough from toxic compounds in order for it to be used in poultry production in Senegal. Indeed, the only deoiling treatment probably did not eliminate enough amounts of phorbol esters as well as antinutritional factors. When this treatment was supplemented by heating, some antinutritional molecules probably could be eliminated. There are mainly the inhibitor of trypsin activity as well as lectin which have been denatured by heat treatment with moist heat (Makkar *et al*., 1998a), with an improvement in the digestibility of proteins (Makkar & Becker, 1998). This was confirmed by Aderibigbe *et al.* (1997); Makkar & Becker (1999); Aregheore *et al*. (2003); Chivandi *et al.* (2006) and Martinez-Herrera *et al*. (2006). However, Adeyemi *et al.* (2001), boiling jatropha seeds, failed to improve dietary intake and weight gains of broiler chickens that received a diet in which peanut meal was gradually substituted by the obtained jatropha. They also found a significant increase in animal mortality according to the incorporation of jatropha.

Trypsin inhibitors prevent the processes of digestion by interfering with the function of proteolytic enzymes in monogastrics (Akande *et al*., 2010), while lectins are known to bind to the intestinal mucosa at the brush border, inducing disruption of nutrient digestibility (Fasina *et al*., 2004). This is also the case for phytates, which cause endogenous mineral and amino acids losses (Cowieson *et al*., 2004), as well as saponins that cause irritation of the mucous membranes and affect the palatability of foods (Cheeke, 1996). However, as regards to phytates and saponins, their concentration in jatropha meal probably is not influenced by heat treatments as shown by Aderibigbe *et al*. (1997) and Martinez-Herrera *et al*. (2006). Moreover, Makkar *et al*. (1988b) showed that roasting did not affect saponin, phytate and was not effective in decreasing lectin activity in the seeds even if trypsine inhibitors were completely inactivated.

Chemical de-oiling supplemented by a heat treatment could not completely eliminate chemical constituents which significantly reduced the animal's appetite for the mixture offered. Makkar *et al.* (1997) had already observed that heat treatment was unable to destroy phorbol esters. Beyond the fact that feed intake is influenced by various factors such as taste, smell and texture (Rolls, 2005), residual concentrations of phorbol esters had also a significant impact on feed intake and on the growth of animals in successive experiments. Areoghore *et al*. (2003) and Pasaribu *et al.* (2009) showed that the

presence of phorbol esters influences feed intake, which is always low. However, on carp, shrimp, trout and pigs as well as on catfish larvae, good growth performance and a good consumption index were obtained with rations incorporating jatropha meal which was treated with organic solvents, followed by heat and simply boiled meal (Harter *et al*., 2011, Kumar *et al*., 2011, Wang *et al*., 2011, Alatise *et al*., 2014, Li *et al*., 2015).

Nesseim *et al*. (article in writing), have accompanied the chemical deoiling by fermentation, using *Aspergillus niger*, preceded and followed by a heat treatment. The product obtained was incorporated as a premix in a commercial ration, made isoproteinic and isoenergetic, partially substituting for peanut meal and distributed to one day-old chicks. The results showed that the treatments undergone by the jatropha kernel probably reduced or even canceled any toxicity. No mortality or signs of intoxication were observed in animals whose diet was incorporating jatropha treated cake compared to those observed in Nesseim *et al.* (article accepted in publication). Feed intake and therefore weight gain was also not influenced by the incorporation of the meal. To our knowledge, such results in poultry are observed for the first time in the litterature.

Jatropha kernel meal is a growth medium and a source of nutrients for microorganisms (Pandey, 2003, Mahanta *et al*., 2008, Ncube *et al*., 2012). These micro-organismes could potentially allow biological detoxification of toxic compounds (Pandey *et al*., 2000), such as phorbol esters (Phengnuam & Suntornsuk, 2013, Bose & Keharia, 2014, Da Luz *et al*., 2014, Hidayat *et al.* , 2014) and anti-nutritional compounds such as phytate, saponins, lectins and trypsin inhibitor (Sharath *et al*., 2014).

Indeed, it has been shown that fermentations of *Jatropha curcas* meal with different species of microorganisms, even if they have not allowed the total removal of toxic and antinutritional compounds, have significantly reduced those (Phengnuam & Suntornsuk, 2013). From this point of view, enzymes produced by microorganisms have played a fundamental role and could be used in subsequent studies. Thus, proteases, phytases and esterases, produced by microorganisms, helped reduce trypsin inhibitors by hydrolysis, phytate by hydrolysis (Reddy & Pierson, 1994; Sumiati *et al*., 2009; Kasuya *et al*., 2013) and esters of phorbol by hydrolysis of the ester linkages (De Barros *et al*., 2011, Najjar *et al*., 2014, Veerabhadrappa *et al*., 2014, Nakao *et al*., 2015). Moreover, Ahmad *et al.* (2000) showed that fermentations with *Aspergillus niger* allowed a phytase production which

improved the apparent availability of calcium and phosphorus and hence the growth parameters of broiler chickens.

This method of detoxification, used by several authors (Belewu *et al*., 2010, Okukpe *et al*., 2010, Wina *et al*., 2010, Belewu *et a*l., 2011, Sanusi *et al*., 2013) despite a reduction of the toxicity of jatropha oil cake, did not significantly improved feed intake, nutrient digestibility and even blood parameters in most animals. However, with rats and Rohu fingerlings, better weight gain was obtained in animals fed with a diet incorporating fermented jatropha meal (Annongu *et al*., 2010b, Saha & Gosh, 2013; Faoziyat *et al*., 2014).

Finally, some natural products such as papain have been used to produce protein isolates from jatropha meal (Selanon *et al.*, 2014). It is a proteolytic enzyme belonging to the cysteine endopeptidase family obtained from papaya latex (*Carica papaya*) (Lieske & Konrad, 1996; Kaul *et al*., 2002), which will cause hydrolysis of the protein molecules (-CO-NH-) groups to give proteoses, peptides and amino acids. It also exhibits esterase activity (Drenth *et al*., 1971). However, feed rations incorporating protein hydrolysates obtained from jatropha oil cake treated with papain did not significantly improve the daily intake of dry matter in the diet and the daily growth in guinea pigs and cockerels (Kouakou *et al*., 2010; Kouadio *et al*., 2016) despite the absence of visible signs of toxicity in animals.

Although the first experiment encourage us to measure animal performance throughout the following essays, an undeniable weakness of this work is the fact that the toxic compounds have not been tested, notably the phorbol esters and the other antinutritional compounds such as phytates, trypsine inhibitors and saponines. In fact, all laboratories contacted in Senegal, do not carry out the quantitative analyzes of these antinutritional compounds. Concerning phorbol esters, we contacted two laboratories which were able to do HPLC analysis but the first one had a software breakdown while the second one, beside its very high costs, requested not only the right information about the analytical protocol but also the required reference values. Hence, a methodology had to be developed in the Department of Analytical Chemistry of the Faculty of Agro-Bio Tech within an interdisciplinary project on the study of the potential and constraints of the use of oil jatropha to produce a biopesticide. An optimization of the phorbol ester assay method was performed by high performance liquid chromatography (HPLC) with standard tetradecanoylphorbol acetate (TPA) solution. The analytical method used had well

confirmed in the samples the presence of compounds within the specific retention time range of phorbol esters. Only the exact amount of phorbol esters present in the flours used in the digestibility tests was difficult to determine given the approximation of the use of TPA as a reference molecule but also the loss of a part of this molecule during the washing with hexane and the possible degradation of the phorbol esters during the storage of the flours. The author also noted that the analysis method used had confirmed the presence of the compounds located within the time frame of the specific retention of the phorbol esters, but could not certify that all the components presents were phorbol esters given the lack of other standards besides the PMA. So, the conclusion that the data obtained from the HPLC analysis were only semi-quantitative and not quantitative (Eylenbosch, 2012). However, the analytical methods currently applied have detection limits of 0.4 - 0.8 and 0.07mg of phorbol esters (TPA equivalent)/kg feed, respectively, by high performance liquid chromatography via an ultraviolet (HPLC-UV) detector and liquid chromatography coupled with mass spectrometry (LC-MS) (EFSA, 2015). These could be used to determine the non-toxic nature of the processed jatropha by-products.

Temperature and humidity influence the dietary intake and thus the weight gain of animals, especially in tropical conditions. To deal with it, the experimental space was designed to offer an optimal thermic comfort to animals in order to minimize the influence of those factors on the physiological parameters. As such, feed allowance was done during the coolest hours of the day, as birds showed decreased consumption during the hottest hours of the day (Donkoh, 1989). Therefore, ambient temperature and humidity were not taken into account because all groups of animals underwent the same environmental conditions. Finally, all experiments were conducted outside the rainy season, which is the hottest and wettest of the year in Senegal.

The tests we carried out were essentially practical and operational in the field. They aimed to answer the question: "Are certain treatments made on jatropha seeds produced in Senegal likely to allow significant and economically profitable production of broiler chicken in the country"? The answer, in the first analysis, is negative, although the fermentation process suggests interesting possibilities.

Further toxicological measurements should have been carried out if it was established that the meal was normally consumed by animals and allowed for some growth. It was found that the by-product was virtually not consumed by poultry, except in the last test. It would therefore have been of little

interest to look for toxic compounds in the meat subsequently produced. Nevertheless, it would have been clearly interesting to establish the toxicological assessment of the various forms of preparation of the jatropha kernel. In the case of the phytate, however, it must be reminded that in the worst situation, the molecule is able to fix only 16% in weight of phosphorus (six atoms per molecule). Owing to a level of phytate in jatropha meal close to 10%, this equals about 1.6% of the meal. In that condition, owing to the low levels of jatropha meal incorporation (4 to 12%), the decrease in total phosphorus availability in the diet was ranging between 0.06 and 0.2 %, i.e., 12 to 38% of phosphorus requirements in poultry diet. Moreover, defatted jatropha meal is known for its very high phosphorus content (more than 1% of meal, see Kumar & Sharma, 2008). And most of the total phosphorus is in the form of phytic acid (Pointillart, 1994). In our opinion, the very poor feed intake and performances observed in our first experiments thus could hardly be ascribed to this molecule but rather to other causes.

However, given that the amount of endogenous phytase, which increase with age, is extremely low in young birds, the diets supplemented with microbial phytase reported present in *Aspergillus niger* (Sebastian *et al*., 1998; Vats & Banerjee, 2002; Selle & Ravindran, 2007) probably resulted in increased digestibility and availability of phytate bound phosphorus in the last experiment.

Whatever, it was interesting to compare the data obtained from these tests with data from the literature, all of which involved poultry fed diets containing jatropha kernel oil cake that had undergone various treatments.

In a first step, a compilation of the results obtained during the various tests that were carried out made it possible to superimpose the feed intake (**Figure 2**) and the average daily gains obtained (**Figure 3**).

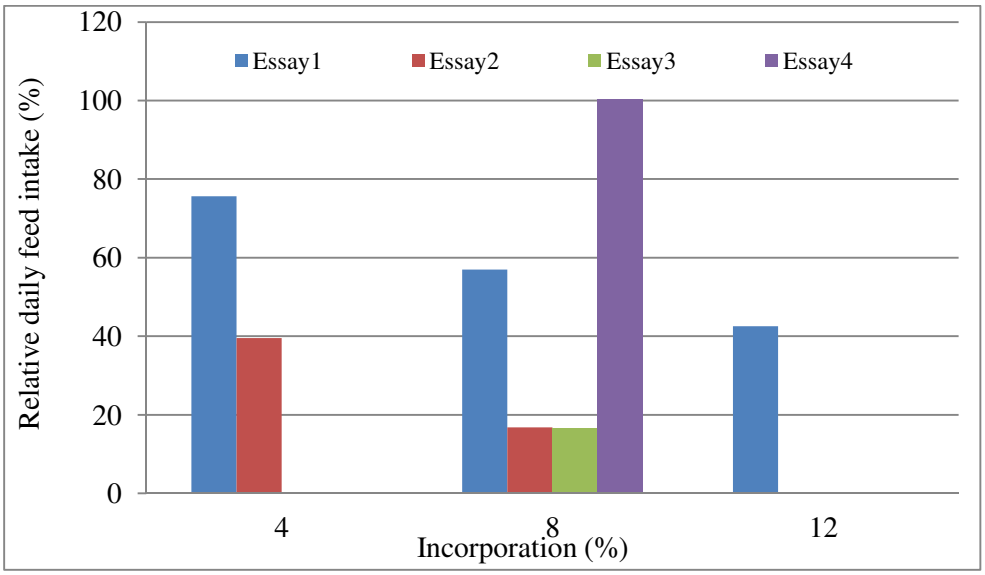



**Figure 2:** Relative daily feed intake with regard to control, in relation to the incorporation rate of jatropha kernel meal.

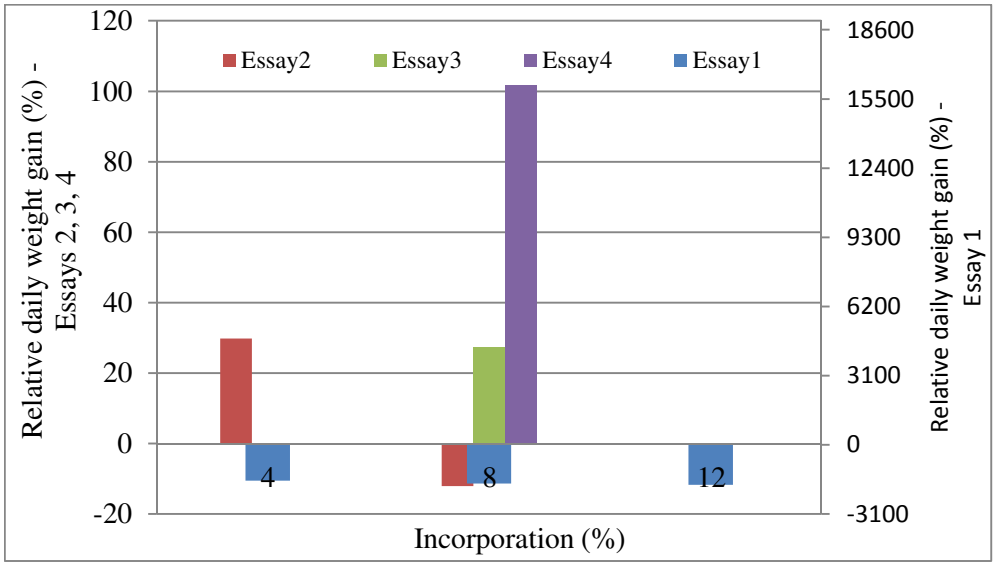



**Figure 3:** Relative daily weight gain with regard to control in relation to the incorporation rate of jatropha kernel meal.

These superpositions of the results have made it possible to demonstrate the impact of the *J. curcas* meal obtained by physical and chemical deoiling with or without thermal or biological treatment and then incorporated into the feeding of poultry at different levels of development on growth parameters. Thus, ingestion and, correlatively, weight gains have decreased almost linealy with the incorporation of the jatropha kernel oil cake for all trials that have been carried out.

The simple physico-chemical treatment of de-oiling of the almond of *J. curcas* did not improve the ingestion and the weight gains in the poultry in which it was observed a decrease of these parameters according to the rate of incorporating the obtained jatropha kernel meal. The additional thermal and then subsequent thermal and biological treatments did not improve these parameters, as is particularly apparent in test 2.

Our compiled results, superimposed on those compiled and obtained by other authors (Chandrasekar *et al*., 2009, Pasaribu *et al*., 2009, Annongu *et al*., 2010a, Sumiati *et al*., 2010, Wina *et al*. 2010, Sumiati *et al*., 2011, Ojediran *et al*., 2014a, Barros *et al*., 2015, Oladunjoye *et al*., 2015, Kouadio *et al*., 2016) allowed us to establish the following comparative evolutions.

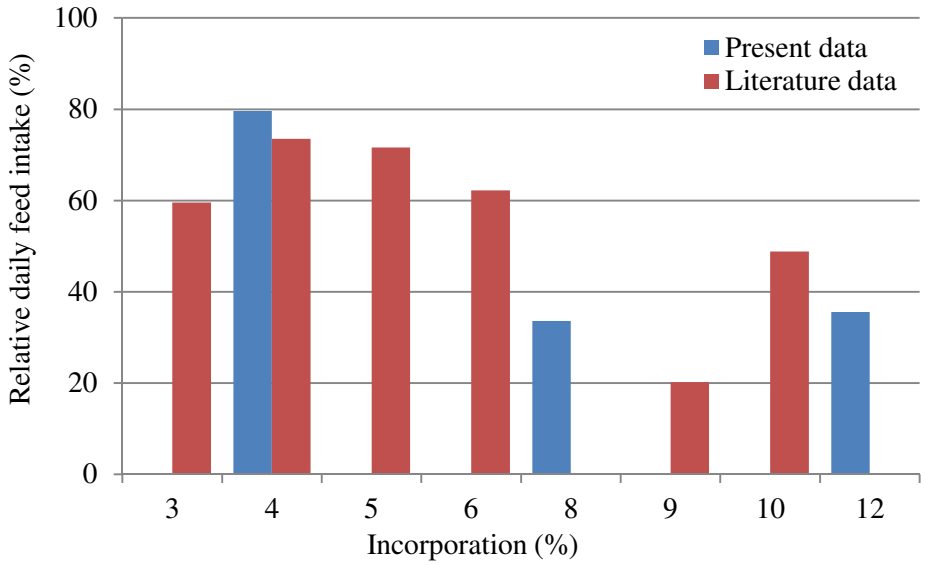



**Figure 4:** Meta-relative daily feed intake with regard to a control group in relation to the incorporation rate of jatropha seed by-products.

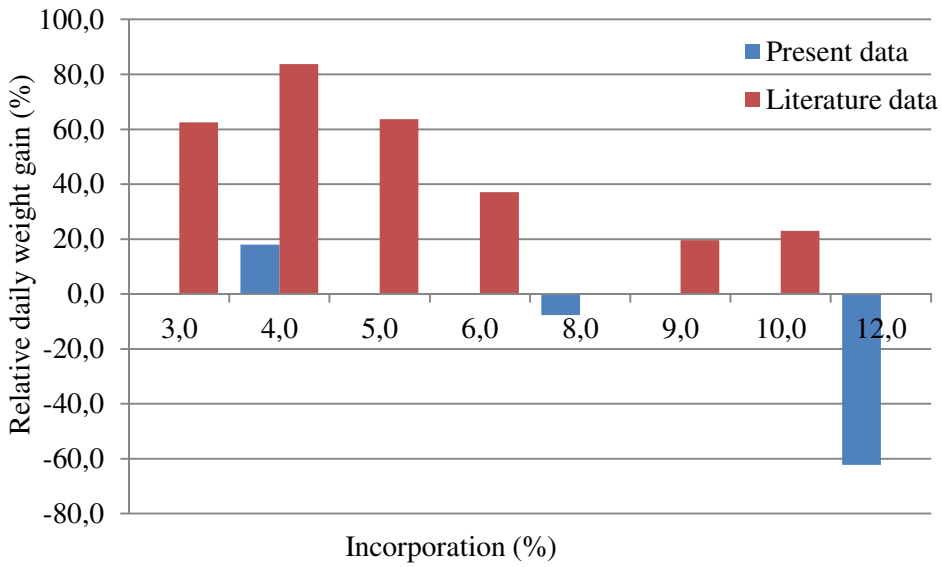



**Figure 5:** Meta-relative daily weight gain with regard to a control group in relation to the incorporation rate of jatropha seed by-products.

As a rule, a good match between the results obtained from the literature and the results obtained from our tests is observed, regardless of the age, sex or race of the animals which have been used in all the experiments. This confirms the absolute impact of the incorporation of jatropha seed by-products on animal performance. The relationship between average daily gain and ingestion with the incorporation rate of jatropha kernel oil cake is generally negative. The performances practically all decreased with the incorporation of jatropha except with the almond biologically treated.

The relative daily weight gain (RADG = ADG / Mean live weight) from the literature studies and from our experiments (**Figure 6**) confirmed the overall negative influence of incorporation of jatropha kernel on growth potential.

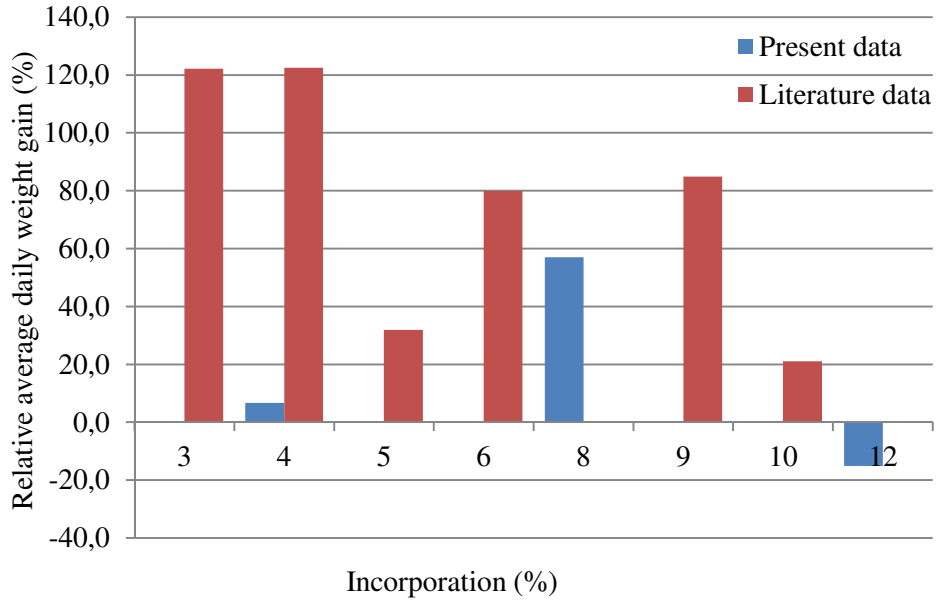



**Figure 6:** Meta-relative daily weight gain relative to a control group in relation to the incorporation rate of jatropha seed by-products.

The statistical analysis according to the generalized linear model (GLM) carried out for the ingestions and the average daily gains as well as for the relative mean daily weight gains shows a decrease of each of the parameters according to the level of incorporation of the jatropha and this in a very significant way ($P<0.01$).

Each of the diets had a more or less pronounced impact on the feed intake, and so, on the weight gain of animals whenever these diets contained jatropha oil cake, despite physical, chemical or chemical treatments or their combination. The biological treatments have, however, improved the feed intake and the weight gains but also significantly reduced the animal mortality by reducing the levls of the toxic and anti-nutritional factors. This shows that the appetite of the animals has been influenced by the still probable presence of antinutritional or toxic compounds which remain present in meals incorporated in diets and which affect feed intake. This observation is surprising in light of the fact that jatropha kernel itself does not seem to have an unpleasant taste, as one of the experimenters tested. It is possible that the negative effect will manifest with some delay. Indeed, it has been observed that the naive animals subjected to the experimental feed normally consumed it, but that repulsion appears in subsequent attempts. The negative experience probably had a negative feedback effect on the intake of the animals as currently observed in poultry (Picard *et al.*, 2000).

On the other hand, weight growth being influenced by feed intake, slowing growth also may be caused by presence of compounds such as curcine or a trypsin inhibitor, especially in young animals (Palacios *et al*., 2004). Furthermore, a decline in chicken performance is probably attributable to appreciable levels of the phorbol esters still bound to the residual lipid fraction of oil cake (Punsuvon *et al*., 2012). In Experiment 4, where the seed sample was totally delipidated by Soxhlet method, negative effects on the growth and decrease of the feed intake were not observed. It is likely that the phorbol esters have been largely removed in the delipidated fraction, in view of the fact that these compounds are partially amphiphilic (Zhang *et al*., 1995). In addition to their interaction with protein kinase C, phorbol esters are capable of releasing proteases, cytokines and activating NADPH oxidase (Goel *et al*., 2007). They can cause damage to the body tissue, causing pain and therefore decreased appetite and feed intake. Curcin and phorbol esters can cause nutrient absorption disorders which causes decreased growth (Annongu *et al*., 2010b). The fermentation of the jatropha kernel meal using *Aspergillus niger* as well as the combination of physico-chemical treatments has, probably, almost fully removed the levels of phorbol esters, thus inducing a better efficiency of the use of proteins and metabolizable feed energy (Sumiati *et al*., 2010). The adverse effects on performance and health of animals that were observed throughout our experiments have been consistent with the results presented by other authors and confirm the limits of use of the jatropha kernel meal in which phorbol esters are considered as the main toxic component. In addition, Berenchtein *et al.* (2014) showed that typical signs of poisoning were not observed when animals ingested feed containing very low amounts of phorbol esters however they had a negative impact on feed intake and average weight gain. Indeed, negative effects of jatropha on the palatability, resulting in a decrease in feed intake was systematically observed on the animals even at the lowest incorporation rates, confirming the observations of Ojediran *et al.* (2014a). However, Widiyastuti *et al.* (2013), incorporating fermented jatropha meal successively with *lactobacillus sp*. and *Bifidobacterium spp*., then mixed with fructooligosaccharides in diets intended for poultry, have tried to influence the palatability of the diet depending on the rate of incorporation but did not improve the ingestion. Furthermore, it was noted that even if mortalities were noted in our experiments, the clinical signs as described by Sirisha *et al.* (2009) have not been identified which may suggest that the phorbol esters that were likely to remain in the diets did not cause severe clinical

signs in animals. The same observations were made by Chandrasekar *et al.* (2009); Ojediran *et al.* (2014a) as well as by Barros *et al.* (2015) who also did not observe significant lesions in the cardiac, hepatic, renal or pulmonary tissues examined. El Badwi *et al.* (1992), El Badwi *et al.* (1995) and Ojediran *et al.* (2014b) reported clinical manifestations and histological lesions, including hepatic lesions (Ojediran *et al.*, 2015) due to incorporation in the diet of processed jatropha kernel meal.

In any case, regardless of its nutritional quality, a feed will not be able to cover the maintenance and growth needs if it is not accepted and ingested by the animals.

The final body weight of the chickens was negatively impacted by incorporation of the jatropha kernel meal. Thus, due to the high toxicity of phorbol esters, jatropha seed oil cake, which contains high protein content but still retains significant quantities of toxic substances, can not be used as a feed ingredient without additional treatments. The latter do not guarantee a significant improvement in animal performance. However fermentation treatments are promising methods of detoxification and deserve to be further studied.

# CONCLUSION GÉNÉRALE

La production d'huile constitue le premier objectif de la culture et de l'exploitation de *Jatropha curcas* L. pour une production d'une gamme de biocarburants. Cette étude a permis de montrer les limites d'utilisation de l'amande de la graine déshuilée en alimentation de poulets. Malgré le niveau nutritionnel de cette ressource, les composés antinutritionnels et toxiques, notamment les esters de phorbol, limitent son utilisation en nutrition animale. Ces derniers, extrêmement toxiques, lipophiles et thermostables, sont partiellement éliminés au cours de l'extraction de l'huile, mais l'efficacité du procédé dépend du type d'extraction. La combinaison de traitements thermiques, chimiques mais aussi biologiques pour détoxifier le tourteau de jatropha a donné de meilleurs résultats, en termes de probable réduction des teneurs en composés toxiques et antinutritionnels. Seulement ces procédés de détoxification n'ont pas permis une valorisation optimale du tourteau de jatropha du fait de la présence résiduelle vraisemblable de ces composés. Les mêmes observations ont été vérifiées par plusieurs auteurs et avec plusieurs espèces animales, mais avec des résultats très inconstants.

Il s'est agit ici d'une première étude aussi large que possible, faite au Sénégal et effectuée par une même équipe sur une même espèce animale.

Le tourteau de jatropha ne s'est pas révélé être une bonne matière première pouvant être incorporée à l'alimentation des monogastriques, notamment des volailles, en substitution aux tourteaux d'arachide ou de soja. Les études sur l'utilisation du tourteau de jatropha ont montré que beaucoup reste à accomplir. En effet, même si la nature des composés toxiques présents dans ce produit est connue, les méthodes d'analyse de ces derniers ont besoin d'être améliorées en vue de l'élaboration d'un procédé de détoxification efficace permettant son utilisation en alimentation animale. Par ailleurs, une fois les processus de déstoxification normalisés, il sera nécessaire de développer un procédé industriel pour réduire ainsi le coût du traitement.

Le tourteau de jatropha pourrait également être étudié et exploité après d'autres processus de traitement tels que la digestion anaérobie définie comme une fermentation microbienne produisant un biogaz contenant principalement du méthane, la gazéification qui est une conversion thermo-chimique produisant des quantités importantes de monoxyde de carbone et de l'hydrogène appelé gaz de synthèse, les amendements organiques en apport nutritionnel pour diverses spéculations culturales, la fermentation en milieu solide pour la production d'enzymes ainsi que l'extraction et la purification de

divers composés ayant un potentiel pharmacologique. Ainsi la production de biodiésel grâce aux sous-produits qu'elle génère, conservera son impact sur l'agriculture et divers aspects de l'industrie.

D'autres études sont nécessaires pour, non seulement, éliminer les facteurs toxiques et antinutritionnels dans une approche économiquement rentable pour une utilisation dans les rations de volailles, mais aussi évaluer avec soin les seuils d'esters de phorbol tolérables dans les régimes alimentaires. Une alternative qui pourrait se révéler prometteuse, au-delà de son coût économique, serait la mise en œuvre de fermentation biologique ou l'utilisation spécifique d'enzymes pour la réduction des esters de phorbol, en particulier. Il faudrait, dans ce dernier cas, mentionner que, du fait de sa teneur en protéines, l'utilisation de concentré de protéines pourrait constituer une autre alternative à un faible coût comparativement aux protéines pouvant être obtenues des cultures vivrières comme le soja ou le blé.

A la différence d'autres graines oléagineuses comme le soja ou le colza, le tourteau de jatropha pourrait difficilement être utilisé dans l'alimentation des animaux à moins de mettre en place, dans une approche collaborative, des cultivars supérieurs à faibles teneurs en facteurs toxiques et antinutritionnels, notamment l'introduction de varitétés dites « non toxiques ».

## RÉFÉRENCES BIBLIOGRAPHIQUES


1. ABDEL GADIR W.S., ONSA T.O., ALI W.E.M., El BADWI S.M.A. et ADAM S.E.I., 2003. Comparative toxicity of *Croton macrostachys*, *Jatropha curcas* and *Piper Abyssinica* seeds in Nubian goats. Small Ruminant Research, **48**(1), 61-67.
2. ABDU-AGUYE I., SANNUSI A., ALAFIYA-TAYO R.A. et BHUSNUMATH S.R., 1986. Acute toxicity studies with *Jatropha curcas* L. Human & Experimental Toxicology, **5**(4), 269-274.
3. ABO EL-FADEL M.H., HUSSEIN A.M. et MOHAMED A.H., 2011. Incorporation *Jatropha curcas* meal on lambs ration and it's effect on lambs performance. Journal of American Science, **7**(2), 129-132.
4. ABOU-ARAB A.A. & ABU-SALEM F.M., 2010. Nutritional quality of *Jatropha curcas* seeds and effect of some physical and chemical treatments on their antinutritional factors. African Journal of Food Science, **4**(3), 93-103.
5. ABU-ARAB A.A. & ABU-SALEM F.M., 2010. Nutritional quality of *Jatropha curcas* seeds and effect of some physical and chemical treatments on their anti-nutritional factors. African Journal of Food Science, **4**(3), 93-103.
6. ACHTEN W., MATHIJS E., VERCHOT L. SINGH V.P. et MUYS B., 2007. Bio-diesel from jatropha: the life-cycle perspective. Expert Seminar on *Jatropha curcas* L. Agronomy and Genetics, Fact Fondation, Wagenigen.
7. ACHTEN W.M.J., VERCHOT L., FRANKEN Y.J., MATHIJS E., SINGH V.P., AERTS R. et MUYS B., 2008. Jatropha bio-diesel production and use. Biomass and Bioenergy, **32**(12), 1063-1084.
8. ADAM S.E.I., 1974. Toxics effects of *Jatropha curcas* in mice. Toxicology, **2**(1), 67-76.
9. ADAM S.E.I. & MAGZOUB M., 1975. Toxicity of *Jatropha curcas* for goats. Toxicology, **4**(3), 388-389.
10. ADERIBIGBE A.O., JOHNSON C.O.L.E., MAKKAR H.P.S., BECKER K. et FOIDL N., 1997. Chemical composition and effect of heat on organic matter -and nitrogen- degradability and some

antinutritional components of jatropha meal. Animal Feed Science and Technology, **67**(2-3), 223-243.

11. ADEYEMI O.A., BALAGUN M.O. et FASINA O.E., 2001. Response of finishing broilers to graded levels of boiled jatropha seeds. The Indian Journal of Animal Sciences, **71**(8), 800-803.
12. AHMAD T., RASOOL S., SARWAR M., HAQ A.U. et HASAN Z.U., 2000. Effect of microbial phytase produced from a fungus *Aspergillus niger* on bioavailability of phosphorus and calcium in broiler chickens. Animal Feed Science a,d Technology, **83**(2), 103-114.
13. AGENCE NATIONALE DE LA STATISTIQUE ET DE LA DEMOGRAPHIE, 2015. Situation économique et sociale du Sénégal en 2012. Ministère de l'Economie, des Finances et du Plan, République du Sénégal, 342p.
14. AHMED O.M. & ADAM S.E.I., 1979. Effects of *Jatropha curcas* on calves. Veterinary Pathology Online, **16**(4), 476-482.
15. AHMED W.A. & SALIMON J., 2009. Phorbol esters as toxic constituents of tropical *Jatropha curcas* seed oil. European Journal of Scientific Research, **31**(3), 429-436.
16. AKANDE K.E., DOMA U.D., AGU H.O. et ADAMU H.M., 2010. Major antinutrients found in plant protein sources: their effect on nutrition. Pakistan Journal of Nutrition, **9**(8), 827-532.
17. AKBAR E., YAAKOB Z., KAMARUDIN S.K., ISMAIL M. et SALIMON J., 2009. Characteristic and composition of *Jatropha curcas* oil seed from Malaysia and its potential as biodiesel feedstock feedstock. European Journal of Scientific Research, **29**(3), 396-403.
18. ALATISE S.P., ADEDOKUN M.A., ADELODUN O.B. et AJIBOYE G.E., 2014. Effects of boiled jatropha kernel meal as a substitute for soybeans meal in diet of African Mud Catfish (*Clarias gariepinus*) fingerlings. Journal of Fisheries and Aquatic Science, **9**, 446-454.
19. ALI N., KURCHANIA A.K. et BABEL S., 2010. Bio-methanisation of *Jatropha curcas* deffated waste. Journal of Engineering and Technology Research, **2**(3), 38-43.
20. ANDERSON-HAFERMANN J.C., ZHANG Y. et PARSONS C.M., 1993. Effects of processing on the nutritional quality of canola meal. Poultry Science, **72**(2), 326-333.
21. ANNONGU A.A., BELEWU M.A. et JOSEPH J.K., 2010a. Potentials of jatropha seeds as substitute protein in nutrition of poultry. Research Journal of Animal Sciences, **4**(1), 1-4.

22. ANNONGU A.A., JOSEPH J.K., APATA D.F., ADEYINA A.O., YOUSUF M.B. et OGUNJIMI K.B., 2010b. Detoxification of *Jatropha curcas* seeds for use in nutrition of monogastric livestock as alternative feedstuff. Pakistan Journal of Nutrition, **9**(9), 902-904.
23. AREGHEORE E.M., BECKER K. et MAKKAR H.P.S., 2003. Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats. The South Pacific Journal of Natural Science, **21**(1), 51-56.
24. AREGHEORE E.M., MAKKAR H.P.S. et BECKER K., 1998. Assessment of lectin activity in a toxic and a non-toxic variety of *Jatropha curcas* using latex agglutination and haemagglutination methods and inactivation of lectin by heat treatments. Journal of the Science of Food and Agriculture, **77**(3), 349-352.
25. AYSSIWEDE S.B., CHRISOSTOME C., OSSEBI W., DIENG A. et HORNICK J.-L., MISSOHOU A., 2010. Utilisation digestive et métabolique et valeur nutritionnelle de la farine de feuilles de *Cassia tora* (Linn.) incorporée dans la ration alimentaire des poulets indigènes du Sénégal. Revue de Médecine Vétérinaire, **161**(12), 549-558.
26. BALAT M., 2011. Potential alternatives to edible oils for biodiesel production – A review of current work. Energy Conversion and Management, **52**(2), 1479-1492.
27. BALDINI M., FERFUIA C., BORTOLOMEAZZI R., VERARDO G., PASCALI J., PIASENTIER E. et FRANCESCHI L., 2014. Determination of phorbol esters in seed and leaves of *Jatropha curcas* in animal tissue by high performance liquid chomatography tandem mass spectrometry. Industrial Crops and Products, **59**, 268-276.
28. BANERJI R., CHOWDHURY A.R., MISRA G., SUDARSANAM G., VERMA S.C. et SRIVASTAVA G.S., 1985. Jatropha seed oils for energy. Biomass, **8**(4), 277-282.
29. BARBIER J., CISSAO M., TACOUROU B., CISSE C., GRAND C. et LOCHT F., 2012. Intérêts de mettre en place une filière courte basée sur la culture du jatropha (*Jatropha curcas*) dans la communauté rurale de Dialacoto. Aide au Développement Gembloux (ADG), Gembloux, 139p.
30. BARROS C.R., RODRIGUES M.A.M., NUNES F.M., KASUYA M.C.M., Da LUZ J.M.R., ALVES A., FERREIRA L.M.M., PINHEIRO V. et MOURÃO J.L., 2015. The effect of *Jatropha*

*curcas* seed on growth performance and internal organs development and lesions in broiler chickens. Revista Brasileira de Ciência Avicola, **17**(SPE), 1-6.

31. BEBAY C.E., 2006. Première évaluation de la structure et de l'importance du secteur avicole commercial et familial en Afrique de l'ouest. Synthèse des rapports nationaux. Organisation des Nations Unies pour l'Alimentation et l'Agriculture, 47p.
32. BECKER K., 2009. Biofuels from *Jatropha curcas* oil - Perspectives for tropical regions. Oléagineux, Corps gras, Lipides, **16**(4), 236-240.
33. BECKER K. & MAKKAR H.P.S., 1998. Effects of phorbol esters in carp (*Cyprinus carpio* L.). Veterinary and Human Toxicology, **40**(2), 82-86.
34. BEERENS P., 2007. Screw-pressing of jatropha seeds for fuelling purposes in less developed countries. Eindhoven University of Technology. Ministerio de Ambiente y Energía-MINAE-. "Plan Nacional de Biocombustibles". Costa Rica, 80p.
35. BELEWU M.A. & AKANDE B.A., 2010. Biological upgrading of the nutritional quality of *Jatropha curcas* kernel cake: effect on performance characteristics of goat. International Research Journal of Biotechnology, **1**(2), 19-22.
36. BELEWU M.A., BELEWU K.Y. et LAWAL I.A., 2011. Cocktail of fungi blend on *Jatropha curcas* kernel cake: effect on feed intake and blood parameters of goat. Libyan Agriculture Research Center Journal International, **2**(3), 138-143.
37. BELEWU M.A., BELEWU K.Y. et OGUNSOLA F.O., 2010. Nutritive value of dietary fungi treated *Jatropha curcas* kernel cake: voluntary intake, growth and digestibility coefficient of goat. Agriculture and Biology Journal of North America, **1**(2), 135-138.
38. BELEWU M.A. & SAM R., 2010. Solid state fermentation of *Jatropha curcas* kernel cake: proximate composition and antinutritional components. Journal of Yeast and Fungal Research, **1**(3), 44-46.
39. BERCHMANS H.J. & HIRATA S., 2008. Biodiesel production from crude *Jatropha curcas* L. seed oil with a high content of free fatty acids. Bioresource Technology, **99**(6), 1716-1721.
40. BERENCHTEIN B., ABDALLA A.L., Do PRADO PAIM T., SBARDELLE M., LOUVANDINI H., FILHO A.L.A., DHANASEKARAN D. et Dos SANTOS P.P., 2014. Effects of detoxified

*Jatropha curcas* kernel meal finishing pig diets on their performance, carcass traits, meat quality and intoxication. Livestock Science, **165**, 100-103.

41. BERHAUT J., 1975. Flore illustrée du Sénégal. Dicotylédones: tome 3. Connaracées à Euphorbiacées. Gouvernement du Sénégal, Ministère du Développement Rural et de l'Hydraulique, Directions des Eaux et Forêts, Dakar, 634p.
42. BEYRA A, DEL CARMEN LEÓN M., IGLESIAS E., FERRÁNDIZ D., HERRERA R., VOLPATO G., GODINEZ D., GUIMARAIS M. et ÁLVAREZ R., 2004. Estudios ethnobotánicos sobre plantas medicinales en la provincial de Camagüey (Cuba). Anales del Jardin Botánico de Madrid, **61**(2), 185-204.
43. BLUMBERG P.M., 1988. Proteine kinase C as the receptor for the phorbol ester tumor promoters: sixth rhoads memorial award lecture. Cancer Research, **48**(1), 1-8.
44. BOSE A. & KEHARIA, 2014. Phorbol ester degradation in jatropha seedcake using white rot fungi. 3 Biotech, **4**(4), 447-450.
45. BRITTAINE R. & LUTALADIO N., 2010. Jatropha: a smallholder bioenergy crop, the potential for Pro-Poor development. Integrated Crop Management, 8, Food and Agriculture Organization of the United Nations, Rome, 96p.
46. CASTAGNA M., TAKAI Y., KAIBUCHI K., SANO K., KIKKAWA U. et NISHIZUKA Y., 1982. Direct activation of calcium-activated, phospholipid-dependant protein kinase by tumor-promoting phorbol esters. Journal of Biologica Chemistry, **257**(13), 7847-7851.
47. CHEEKE P.R., 1996. Biological effects of feed and forage saponins and their impacts on animal production. *In*: Saponins Used in Food and Agriculture. Springer US, **405**, 377-385.
48. CHANDRASEKAR S., RAVINDER REDDY V. et RAJASHEKHAR REDDY A., 2009. Effect of feeding differently processed jatropha (*Jatropha curcas*) cake on the performance of broilers. Indian Journal of Poultry Science, **44**(3), 352-357.
49. CHIVANDI E., ERLWANGER K.H., MAKUZA S.M., READ J.S. et MTIMUNI J.P., 2006. Effects of dietary *Jatropha curcas* meal on percent packed cell volume, serum glucose, cholesterol and triglyceride concentration and alpha-amylase activity of weaned fattening pigs. Research Journal of Animal and Veterinary Sciences, **1**(1), 18-24.

50. CHIVANDI E., MAKUZA S.M., ERLANDER K.H., MTIMUNI J.P., READ J.S. et TIVAPASI M., 2000. Effects of dietary *Jatropha curcas* on the haematology of weaned pigs. Zimbabwe Veterinary Journal, **31**(4), 83-91.

51. CHIVANDI E., MTIMUNI J.P., READ J.S. et MAKUZA S.M., 2004. Effect of processing method on phrobol ester concentration, total phenolics, trypsin inhibitor and the proximate composition of the Zimbabwean *Jatropha curcas* provenance: a potential livestock feed. Pakistan Journal of Biological Sciences, **7**(6), 1001-1005.

52. COTHENET G. & BASTIANELLI D., 1999. Les matières premières disponibles pour l'alimentation des volailles en zones chaudes. *In*: La production de poulets de chair en climat chaud, Paris : ITAVI, 60-70.

53. COWIESON A.J., ACAMOVIC T. et BEDFORD M.R., 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. British Poultry Science, **45**(1), 101-108

54. DAHOUDA M., TOLEBA S.S., YOUSSAO A.K.I., MAMA ALI A.A., AHOUNOU S. et HORNICK J.-L., 2009. Utilisation des cossettes et des feuilles de manioc en finition des pintades (*Numida meleagris* L.): performances zootechniques, coûts de production, caractéristiques de la carcasse et qualité de la viande. Annales de Médecine Vétérinaire, **153**, 82-87.

55. DALE N., 1996. Variation in feed ingredient quality: oilseeds meals. Animal Feed Science and Technology, **59**(1-3), 129-135.

56. Da LUZ J.M.R., NUNES M.D., PAES S.A., TORRES D.P. et KASUYA M.C.M., 2014. Bio-detoxification of *Jatropha curcas* seed cake by *Pleurotus ostreatus*. African Journal of Microbiology Research, **8**(11), 1148-1156.

57. DAS M., UPPAL H.S., SINGH R., BERI S., MOHAN K.S., GUPTA V.C. et ADHOLEYA A., 2011. Co-composting of physic nut (*Jatropha curcas*) deoiled cake with rice straw and different animal dung. Bioresource Technology, **102**(11), 6541-6546.

58. Da SILVA L.D., PEREIRA O.G., VALADARES FILHO S.C., RIBEIRO K.G., VALADARES R.F.D., SILVA T.C. et SANTOS S.A., 2015. Intake, digestibility, and nitrogen efficiency in

Holstein heifers fed treated jatropha (*Jatropha curcas* L.) kernel cake. Livestock Science, **178**, 100-107.

59. De BARROS C.R., FERREIRA L.M., NUNES F.M., BEZERRA R.M., DIAS A.A., GUEDES C.V., CONE J.W., MARQUES G.S.M. et RODRIGUES A.M., 2011. The potential of white-rot fungi to degrade phorbol esters of *Jatropha curcas* L. seed cake. Engineering in Life Science, **11**(1), 107-110.
60. DEHGAN B. & WEBSTER G.L., 1979. Morphology and infrageneric relationships of the genus *Jatropha* (*Euphobiaceae*). University of California Publications in BOTANY, 74, Los Angeles, 73p.
61. DEVAPPA R.K. & SWAMYLINGAPPA B., 2008. Biochemical and nutritional evaluation of jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors. Journal of the Science of Food and Agriculture, **88**(5), 911-919.
62. DEVAPPA R.K., MAES J., MAKKAR H.P.S., De GREYT W. et BECKER K., 2010. Quality of biodiesel prepared from phorbol ester extracted *Jatropha curcas* oil. Journal of the American Oil Chemists' Society, **87**(6), 697-704.
63. DEVAPPA R.K., MAKKAR H.P.S. et BECKER K., 2010. Biodegradation of *Jatropha curcas* phorbol esters in soil. Journal of the Science of Food and Agriculture, **90**(12), 1090-1097.
64. DEVAPPA R.K. & SWAMYLINGAPPA B., 2008. Biochemical and nutritional evaluation of jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors. Journal of the Science of Food and Agriculture, **88**(5), 911-919.
65. DIAW M.T., DIENG A., MERGEAI G., CAMARA A. et HORNICK J.-L., 2012. Effet de la substitution totale du tourteau d'arachide par la fève de coton glandless sur les performances zootechniques de poulets de chair au Sénégal. Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, **65**(1-2), 17-23
66. DIVAKARA B.N., UPADHYAYA H.D., WANI S.P. et LAXMIPATHI GOWDA C.L., 2010. Biology and genetic improvement of *Jatropha curcas* L.: a review. Applied Energy, **87**(3), 732-742.

67. DONKOH A., 1989. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. International Journal of Biometeorology, **33**(4), 259-265.

68. DRENTH J., JANSONIUS J.N., KOEKOEK R. et WOLTHERS B.G., 1971. The structure of papain. Advances in Protein Chemistry, **25**, 79-115.

69. EFSA CONTAM Panel (EFSA Panel on contaminants in the food chain), 2015. Scientific opinion on risks for human and animal health related to the presence of phorbol esters in jatropha kernel meal. EFSA Journal, **13**(12), 4321, 80p.

70. El BADWI S.M. et ADAM S.E., 1992. Toxic effects of low levels of dietary *Jatropha curcas* seed on brown Hisex chicks. Veterinary and Human Toxicology, **34**(2), 112-115.

71. El BADWI S.M., ADAM S.E.I. et HAPKE H.J., 1995. Comparative toxicity of *Ricinus communis* and *Jatropha curcas* in Brown Hisex chicks. Deutsche Tierarztliche Wochenschrift, **102**(2), 75-77.

72. El DIWANI G.I., EL RAFEI S.A. et HAWASH S.I., 2011. Ozone for phorbol esters removal from Egyptian jatropha oil seed cake. Advances in Applied Science Research, **2**(4), 221-232.

73. EMIL A., YAAKOB Z., KUMAR M.N.S., JAHIM J.M. et SALIMON J., 2010. Comparative evaluation of physicochemical properties of jatropha seed oil from Malaysia, Indonesia and Thailand. Journal of the American Oil Chemists' Society, **87**(6), 689-695.

74. ENUJIUGHA V.N. & AYODELE-ONI O., 2003. Evaluation of nutrients and some anti-nutrients in lesser known, underutilized oilseeds. International journal of Food Science & Technology, **38**(5), 525-528.

75. EYLENBOSCH D., 2012. Evaluation de l'effet insecticide de l'huile de *Jatropha curcas* L. sur les ravageurs du chou pommé et quantification des esters de phorbol presents au sein de cette huile. Travail de Fin d'Etudes présenté en vue de l'obtention du diplôme de Master Bioingénieur en Sciences Agronomiques, Gembloux Agro-Bio Tech, Université de Liège, 80p.

76. EZE S.O.O., 2016. Pysico-chemical properties of oil from some selected underutilized oil seeds available for biodiesel preparation. African Journal of Biotechnology, **11**(2), 10003-10007.

77. FAOZIYAT S.A., AMINA A.E.I., ADEYEMO A.A., MUHAMMED R., SULAIMAN A.M., ALIYU A.O. et ADEYEMI O.S., 2014. *Aspergillus*-fermented *Jatropha curcas* seed cake:

proximate composition and effects on biochemical indices in Wistar rats. Biological letters, **51**(1), 37-46.

78. FASINA Y.O., GARLICH J.D., CLASSEN H.L., FERKET P.R., HAVENSTEIN G.B., GRIMES J.L., QURESHI M.A. et CHRISTENSENT V.L., 2004. Response of turkey poults to soybean lectin levels typically encountered in commercial diets. 1. Effects on growth and nutrient digestibility. Poultry Science, **83**(9), 1559-1571.
79. FELIX-BERNAL J.A., ANGULO-ESCALANTE M.A., ESTRADA-ANGULO A., HEREDIA J.B., MUY-RANGEL D., LÓPEZ-SOTO M.A., BARRERAS A. et PLASCENCIA A., 2014. Fedding value of non-toxic *Jatropha curcas* seed cake for partially replacing dry-rolled corn and soybean meal in lambs fed finishing diets. Animal Feed Science and Technology, **198**, 107-116.
80. FERREIRA O.R., BRITO S.S., LIMA F.G., MARIANO SOUZA D.P., MENDOCA S., RIBEIRO J.A.A., MAIORKA P.C., ARAÚJO V.L., NEIVA J.N.M., FIORAVANTE M.C.S., RAMOS A.T. et MARUO V.M., 2012. *Jatropha curcas* pericarp toxicity in sheep. Archivo Brasileiro de Medicina Veterinária e Zootechnica, **64**(3), 559-567.
81. FRANCIS G., EDINGER R. et BECKER K., 2005. A concept for simultaneous wasteland reclamation, fuel production, and socio-economic development in degraded areas in India: need, potential and perspectives of jatropha plantations. Natural Resources Forum, **29**, 12-24.
82. FRANCIS G., KEREM Z., MAKKAR H.P.S. et BECKER K., 2002. The biological action of saponins in animal systems: a review. British Journal of Nutrition, **88**(06), 587-605.
83. FRANCIS G., MAKKAR H.P.S. et BECKER K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, **199**(3-4), 197-227.
84. GUEDES R.E., De ALMEIDA CRUZ F., De LIMA M.C. et MENDES M.F., 2014. Detoxification of *Jatropha curcas* seed cake using chemical treatment: analysis with a central composite rotatable design. Industrial Crops and Products, **52**, 537-543.
85. GHANDI V.M., CHERIAN K.M. et MULKY M.J., 1995. Toxicological studies on ratanjyot oil. Food and Chemical Toxicology, **33**(1), 39-42.
86. GOEL G., MAKKAR H.P.S., FRANCIS G. et BECKER K., 2007. Phorbol esters: structure, biological activity, and toxicity in animals. International Journal of Toxicology, **26**(4), 279-288.

87. HAAS W. & MITTELBACH M., 2000. Detoxification experiments with the seed oil from *Jatropha curcas* L. Industrial Crops and Products, **12**(2), 111-118.

88. HAJOS G., GELENCSER E., PUSZTAI A., GRANT G. SAKHRI M. et BARDOCZ S., 1995. Biological effects and survival of trypsin inhibitors and the agglutinin from soybean in the small intestine of the rat. Journal of Agricultural and Food Chemistry, **43**(1), 165-170.

89. HARTER T., BUHRKE F., KUMAR V., FOCKEN U., MAKKAR H.P.S. et BECKER K., 2011. Substitution of fish meal by *Jatropha curcas* kernel meal: effects on growth performance and body composition of white leg shrimp (*Litopenaeus vannamei*). Aquaculture Nutrition, **17**(5), 542-548.

90. HELLER J., 1996. Physic nuts. *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops. 1., Rome: Institute of Plant genetics and Crop Plant research, gatersleben/International Plant genetic resources Institute, 66p.

91. HENNING R.K., 2003. The Jatropha booklet – a guide to the jatropha system and its dissemination in africa. baganí gbr.- 37p.

92. HENNING R.K., 2008. Identification, selection and multiplication of high yielding *Jatropha curcas* L. plants and economic key points for viable jatropha oil production costs. International Consultation on Pro-poor jatropha Development, Rome IFAD, 10-11.

93. HIDIYA C., HASTUTI P., WARDHANI A.K. et NADIA L.S., 2014. Method of phorbol ester degradation on *Jatropha curcas* L. seed cake using rice bran lipase. Journal of Bioscience and Bioengineering, **117**(3), 372-374.

94. JONGSCHAAP R., CORRE W., BINDRABAN P.S. et BRANDENBURG W.A., 2007. Claims and facts on *Jatropha curcas* L. Plant Research International B.V., Wageningen, 42 p.

95. KASUYA M.C.M., Da LUZ J.M.R., PEREIRA L.P.D., Da SILVA J.S., MONTAVANI H.C. et RODRGUES M.T., 2013. Bio-detoxification of jatropha seed cake and its use in animal feed. *In*: Biodiesel – Feedstock, production and applications, *Edited by* Zhen Fang, *In Tech Publisher*, 498p.

96. KATOLE S., SAHA S.K., SASTRY V.R.B., LADE M.H. et PRAKASH B., 2011. Intake, blood metablites and hormonal profile in sheep fed processed jatropha (*Jatropha curcas*) meal. Animal Feed Science and Technology, **170**(1-2), 21-26.

97. KAUL P., SATHISH H.A. et PRAKASH V., 2002. Effect of metal ions on the structure and activity of papain from *Carica papaya*. Nahrung/Food, 46(1), 2-6.

98. KHEIRA A.A.A. & ATTA N.M.M., 2009. Response of *Jatropha curcas* L. to water deficit: yield, water use efficiency and oilseed characteristics. Biomass and Bioenergy, **33**(10), 1343-1350.

99. KING A.J., HE W., CUEVAS J.A., FREUDENBERGER M., RAMIARAMANANA D. et GRAHAM I.A., 2009. Potential of *Jatropha curcas* as a source of renewable oil and animal feed. Journal of Experimental Botany, **60**(10), 2897-2905.

100. KOCHHAR S., SINGH S.P. et KOCHHAR V.K., 2008. Effects of auxins and associated biochemical changes during clonal propagation of the biofuel plant – *Jatropha curcas*. Biomass and Bioenergy, **32**(12), 1136-1143.

101. KONGMANY S., FURUTA M., MATSUURA H., OKUDA S., IMAMURA K. et MAEDA Y., 2014. Degradation of phorbol 12, 13-diacetate in aqueous solution by gamma irradiation. Radiation Physics and Chemistry, **105**, 98-103.

102. KOUADIO K.B., DOUGNOU M.G. et KOUAKOU N.D.V., 2016. Effet de la supplémentation de l'aliment croissance des coquelets (Warren) par du tourteau de *Jatropha curcas* détoxifié. International of Animal & Plant Sciences, **28**(3), 4479-4487.

103. KOUAKOU N.D.V., THYS E., ASSIDJO E.N. et GRONGNET J.F., 2010. Ingestion et digestibilité *in vivo* du Panicum maximum associé à trois compléments : tourteau de Jatropha curcas, tourteau de coton (*Gossipium hirsutum*) et *Euphorbia heterophylla* chez le cobaye (*Cavia porcellus* L.). Tropicultura, **28**(3), 173-177.

104. KUMAR V., MAKKAR H.P.S. et BECKER K., 2011. Detoxified *Jatropha curcas* kernel meal as a dietary protein source: growth performance, nutrient utilization and digestive enzymes in common carp (*Cyprinus carpio* L.) fingerlings. Aquaculture Nutrition, **17**(3), 313-326.

105. KUMAR A. & SHARMA S., 2008. An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): a review. Industrial Crops and Products, **28**(1), 1-10.

106. KUREEL R.S., 2006. Prospects and potential of *Jatropha curcas* for biodiesel production. *In*: Biodiesel Conference towards Energy Independence – Focus on Jatropha. Papers presented at the Conference Rashtrapati Nilayam, Bolaram, Hyderabab, 43-74.

107. LAGO R.C.A., 2009. Castor and jatropha oils: production strategies - A review. Oilseed & Fats Crops and Lipids, **16**(4-5-6), 241-247.

108. LI Y., CHEN L., LIN Y., FANG Z.F., CHE L.Q., XU S.Y. et WU D., 2015. Effects of replacing soybean meal with detoxified *Jatropha curcas* kernel meal in the diet on growth performance and histopathological parameters of growing pigs. Animal Feed Science and Technology, **204**, 18-27.

109. LI W., LI J., LI L., LU D. et CHEN F., 2011. Extarction of phorbol esters from *Jatropha curcas* seeds and their insecticidal activities against *Pieris rapae* larvae. Chinese Journal of Applied & Environmental Biology, **17**, 532-536.

110. LIBERALINO A.A.A., BAMBIRRA E.A., MORAES-SANTOS T. et VIEIRA E.C., 1988. *Jatropha curcas* L. seeds: chemical analysis and toxicity. Arquivos de Biologia e Tecnologia, **31**(4), 539-550.

111. LIESKE B. & KONRAD G., 1996. Physico-chemical and functional properties of whey protein as affected by limited papain proteolysis and selective ultrafiltration. International Dairy Journal, **6**(1), 13-31.

112. LIN J., YAN F., TANG L. et CHEN F., 2003. Antitumor effects of curcin from seeds of *Jatropha curcas*. Acta Pharmacologica Sinica, **24**(3), 241-246.

113. LLOPIS J., BOZA J., GONZALEZ-MOLÈS A. et LUQUE J.A., 1981. Etude des possibilités d'emploi de la drêche de brasserie dans l'alimentation des monogastriques. I. Expériences chez des rats et des poulets, concernant la qualité nutritive de la protéine de deux fractions de la drêche de brasserie. Annales de Zootechnie, **30**(1), 77-85.

114. MAHANTA N., GUPTA A. et KHARE S.K., 2008. Production of protease and lipase by solvent tolerant *Pseudomonas aeruginosa* PseA in solid-state fermentation using *Jatropha curcas* seed cake as substrate. Bioresource Technology, **99**(6), 1729-1735.

115. MAKKAR H.P.S., 2016. State-of-the-art on detoxification of *Jatropha curcas* products aimed for use as animal and fish feed: a review. Animal Feed Science and Technology, **222**, 87-99.

116. MAKKAR H.P.S., ADERIBIGBE A.O. et BECKER K., 1998a. Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. Food Chemistry, **62**(2), 207-215.

117. MAKKAR H.P.S. & BECKER K., 1998. *Jatropha curcas* toxicity: identification of toxic principle(s). Toxic Plants and Other Natural Toxicants, 554-558.

118. MAKKAR H.P.S. & BECKER K., 1999. Nutritional studies on rats and fish (carp *Cyprinus carpio*) fed diets containing unheated and heated *Jatropha curcas* meal of a non-toxic provenance. Plant foods for Humans Nutrtion, **53**(3), 183-192.

119. MAKKAR H.P.S. & BECKER K., 2009. *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. European Journal of Lipid Science and Technology, **111**(8), 773-787.

120. MAKKAR H.P.S, BECKER K., NITIS I.M. et SHIN M.T., 1999. Plants toxins and detoxication methods to improve feed quality of tropical seeds. Asian-Australian Journal of Animal Science, **12**(3), 467-480.

121. MAKKAR H.P.S., BECKER K. et SCHMOOK B., 1998b. Edible provenances of *Jatropha curcas* from Quintana Roo state of Mexico and effect of roasting and antinutrient and toxic factors in seeds. Plant Foods for Humans Nutrition, **52**(1), 31-36.

122. MAKKAR H.P.S., BECKER K., SPORER F. et WINK M., 1997. Studies on nutritive potential and toxic contituents of different provenances of *Jatropha curcas*. Journal of Agricultural and Food Chemistry, **45**(8), 3152-3157.

123. MAKKAR H.P.S., FRANCIS G. et BECKER K., 2008. Protein concentrate from *Jatropha curcas* screw-pressed seed cake and toxic and antinutritional factors in protein concentrate. Journal of the Science of Food and Agriculture, **88**(9), 1542-1548.

124. MAKKAR H.P.S, MAES J., De GREYT W. et BECKER K., 2009. Removal and degradation of phorbol esters during pre-treatment and transesterification of *Jatropha curcas* oil. Journal of the American Oil Chemists' Society, **86**(2), 173-181.

125. MAMPANE K.J., JOUBERT P.H. et HAY I.T., 2006. *Jatropha curcas*: use as a traditional Tswana medicine and its role as a cause of acute poisoning. Phytotherapy Research, **1**(1), 50-51.

126. MARTINEZ-HERRERA J., MARTINEZ C.J., AYALA A.M., SICILIANO L.G., ESCOBEDO R.M., ORTIZ G.D., CEVALLOS G.C., MAKKAR H.P.S., FRANCIS G. et BECKER K., 2012. Evaluation of the nutritional quality of nontoxic kernel flour from *Jatropha curcas* L. in rats. Journal of Food Quality, **35**(2), 152-158.

127. MARTINEZ-HERRERA J., SIDDHURAJU P., FRANCIS G., DAVILA-ORTIZ G. et BECKER K., 2006.Chemical composition, toxic/antimetabolic constituents, and effects of differents treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. Food Chemistry, **96**(1), 80-89

128. McKEVITH B., 2005. Nutritional aspects of oilsseds. Nutrition Bulletin, **30**(1), 13-26.

129. MEDZA MVE S.D., MERGEAI G., BAUDOIN J.-P. et TOUSSAINT A., 2010. Amélioration du taux de multiplication *in vitro* de *Jatropha curcas* L. Tropicultura, **28**(4), 200-204.

130. MEITE A., KOUAME K.G. et KATI-COULIBALY S., 2006. Lectines : substances antinutritionelles ? Médecine et Nutrition, **42**(4), 179-187.

131. MINISTERE DU DEVELOPPEMENT RURAL ET DE L'AGRICULTURE, 2007. Programme spécial biocarburants. République du Sénégal, 24p.

132. MINISTERE DE L'ELEVAGE ET DES PRODUCTIONS ANIMALES, 2014. Rapport d'activités 2013. République du Sénégal, 66p.

133. MONTOYA J.L.D. & TEJEDA E.P., 1989. Potential multipurpose agro forestry crop identified for the Mexican tropics. *In*: New crops for food in industry, G. E. Wickens, N. Haq and P. Day, Chapman and Hall Ltd, London, 447 p.

134. NAJJAR A., ABDULLAH N., SAAD W.Z., AHMAD S., OUSKOUEIAN E., ABAS F. et GHERBAWY Y., 2014. Detoxification of toxic phorbol esters from Malaysian *Jatropha curcas* Linn. Kernel by *Trichoderma spp*. and endophytic fungi. International Journal of Molecular Sciences, **15**(2), 2274-2288.

135. NAKAO M., HASEGAWA G., YASUHARA T. et ISHIHARA Y., 2015. Degradation of *Jatropha curcas* phorbol esters derived from jatropha oil cake and their tumor-promoting activity. Ecotoxicology and Environmental Safety, **114**, 357-364.

136. NAVARRO-PINEDA F.S., BAZ-RODRIGUEZ S.A., HANDLER R. et SACRAMENTO-RIVERO J.C., 2016. Advances on processing of *Jatropha curcas* towards a whole-crop biorefinery. Renewable and Sustainable Energy Reviews, **54**, 247-269.

137. NCUBE T., HOWARD R.L., ABOTSI E.K., JANSEN E.L., Van RENSBURG J. et NCUBE I., 2012. *Jatropha curcas* seed cake as substrate for production of xylanase and cellulase by *Aspergillus niger* FGSCA733 in solid-state fermentation. Industrial Crops and Products, **37**(1), 118-123.

138. NDIR K.N., KANE M., OUATTARA B., BAYALA R. et DIEDHIOU I., 2013. Variability in seed traits, oil content and genetic diversity in local and exotis accessions of *Jatropha curcas* L. in Senegal. African Journal of Biotechnology, **12**(34), 5267-5277.

139. NESSEIM T.D.T., DIENG A., MERGEAI G., NDIAYE S. et HORNICK J.-L., 2015. Digestibility of solvent-treated *Jatropha curcas* kernel by broiler chickens in Senegal. Tropical Animal Health and Production, **47**(8), 1553-1560.

140. ODUSOTE O.M., ABIOYE A.O. et ROTIBI M.O., 2002. *Jatropha curcas* seed oil Linn (*Euphorbiaceae*): contraceptive activity and an oral formulation. Nigerian Quarterly Journal of Hospital Medicine, **12**(1), 44-47.

141. OLADUNJOYE I.O., OJEDIRAN T., ARINGBANGBA C., AKINRINLA O.S. et OPAKUNLE O.G., 2014. Effects of inclusion level and length of fermentation on the utilization of jatropha (*Jatropha curcas*) seed cake by broiler chickens. International Journal of Current Microbiology and Applied Sciences, **3**(7), 44-54.

142. OJEDIRAN T.K., ADISA Y.A., YUSUF S.A. et EMIOLA I.A., 2014a. Nutritional evaluation of processed *Jatropha curcas* kernel meals: effects on growth performance of broiler chicks. Journal of Animal Science Advances, **1**(11), 1110-1121.

143. OJEDIRAN T.K., ALAMU D., OLAYENI T. et EMIOLA A., 2015. Hepatic histology of broiler chicks fed differently processed *Jatropha curcas* kernel meals. Global Journal of Animal Scientific Research, **3**(4).

144. OJEDIRAN T.K. & EMIOLA I.A., 2014b. Toxicological impact of processed *Jatropha curcas* meals on the gut morphology and kidney of broiler chicks. Journal of Animal Science Advances, **1**(4), 1122-1131.

145. OJO R.J., OGUCHE P.I., KUBE G.D & UDZER T.E., 2013. Effect of *Jatropha curcas* supplemented diet on broilers. Scholars Academic Journal of Biosciences, **1**(6), 329-336.

146. OKUKPE K.M., BELEWU M.A. et BADMOS A.H.A., 2010. Growth and perfomance characteristics of West African dwarf goats fed *Trichoderma* treated *Jatropha curcas* seed cake. Proceeding 35th Conference, Nigerian Society for Animal Production, Ibadan, 608-611.

147. OSKOUEIAN E., ABDULLAH N. et AHMAD S., 2012. Phorbol esters insolated from jatropha meal induced apoptosis-mediated inhibition in proliferation of chang and vero cell lines. International Journal of Molecular Sciences, **13**(11), 13816-13829.

148. OSONIYI O. & ONAJOBI F., 2003. Coagulant and anticoagulant activities in *Jatropha curcas* latex. Journal of Ethnopharmacology, **89**(1), 101-105.

149. OUATTARA B., NDIR K.N., GUEYE M.C., DIEDHIOU I., BARNAUD A., FONCEKA D., CISSE N., AKPO E.L. et DIOUF D., 2014. Genetic diversity of *Jatropha curcas* L. in Senegal compared with exotic accessions based on microsatellite markers. Genetic Resources and Crop Evolution, **61**(6), 1039-1045.

150. OUATTARA B., DIEDHIOU I., NDIR K.N., AGBANGBA E.C., CISSE N., DIOUF D., AKPO E.L. et ZONGO J.D., 2013. Variation in seed traits and distribution of *Jatropha curcas* L. in Senegal. International Journal of Current Research, **5**(2), 17-21.

151. PALACIOS M.F., EASTER R.A., SOLTWEDEL K.T., PARSONS C.M., DOUGLAS M.W., HYMOWITZ T. et PETTIGREW J.E., 2004. Effect of soybean variety and processing on growth performance of young chicks and pigs. Journal of Animal Science, **82**(4), 1108-1114.

152. PANDEY A., 2003. Solid-state fermentation. Bichemical Engineering Journal, **13**(2-3), 81-84.

153. PANDEY A., SOCCOL C.R. et MITCHELL D., 2000. New developments in solid state fermentation: I-bioprocesses and products. Process Biochemistry, **35**(10), 1153-1169.

154. PARAWIRA W., 2010. Biodiesel production from *Jatropha curcas*: A review. Scientific Research and Essays, **5**(14), 1796-1808.

155. PASARIBU T., WINA E., TANGENDJAJA B. et ISKANDAR S., 2009. Performance of broiler chicken fed physically and chemically treated jatropha (*Jatropha curcas*) seed meal. Indonesian Journal of Animal and Veterinary Sciences, **14**(1), 11-18.

156. PATOLIA J.S., GHOSH A., CHIKARA J., CHAUDHARY D.R., PARMAR D.R. et BHUVA H.M., 2007. Response of *Jatropha curcas* grown on wasteland to N and P fertilization. Prospects for jatropha methyl ester (biodiesel) in India. International Journal of Environment Studies, **64**(6), 659-674.

157. PEACE O.E. & ALADESANMI A.O., 2008. Effect of fermentation on some chemical and nutritive properties of berlandier nettle spurge (*Jatropha cathartica*) and physic nut (*Jatropha curcas*) seeds. Pakistan Journal of Nutrition, **7**(2), 292-296.

158. PHASUKARRATCHAI N., TONTAYAKOM V. et TONGCUMPOU C., 2012. Reduction of phorbol esters in *Jatropha curcas* L. pressed meal by surfactant solutions extraction. Biomass and Bioenergy, **45**, 48-56.

159. PHENGNUAM T. & SUNTORNSUK W., 2013. Detoxification and anti-nutrients reduction of *Jatropha curcas* seed cake by *Bacillus* fermentation. Journal of Bioscience and Bioengineering, **115**(2), 168-172.

160. PICARD M., Le FUR C., MELCION J.P. et BOUCHOT C., 2000. Caractéristiques granulométriques de l'aliment : le « point de vue » (et de toucher) des volailles. INRA Production Animale, **13**(2), 117-130.

161. POINTILLART A, 1994. Phytates, phytases : leur importance dans l'alimentation des monogastriques. INRA Productions Animales, **7**(1), 29-39.

162. PRADHAN R.C., NAIK S.N., BHATNAGAR N. et VIJAY V.K., 2009. Moisture-dependent physical properties of jatropha fruit. Industrial Crops and Products, **29**(2-3), 341-347.

163. PUNSUVON V., NOKKAEW R. et KARNASUTA S., 2012. Determination of toxic phorbol esters in biofertilizer produced with *Jatropha curcas* seed cake. Science Asia, **38**, 223-225.

164. RAHMA E.H., MANSOUR E.H. et HAMODA S.T., 2013. Biological evaluation of *Jatropha curcas* seed as a new source of protein. Merit Research Journal of Food Science and Technology, **1**(2), 23-30.

165. RAKSHIT K.D., DARUKESHWARA J., RATHINA RAJ K., NARASIMHAMURTHY K., SAIBABA P. et BHAGYA S., 2008. Toxicity studies of detoxified jatropha meal (*Jatropha curcas*) in rats. Food and Chemical Toxicology, **46**(12), 3621-3625.

166. RAVINDRAN V., CABAHUG S., RAVINDRAN G., SELLE P.H. et BRYDEN W.L., 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. British Poultry Science, **41**(2), 193-200.

167. REDDY N.R. & PIERSON M.D., 1994. Reduction in antinutritional and toxic components in plants foods by fermentation. Food Research International, **27**(3), 281-290.

168. ROLLS E.T., 2005. Taste, olfactory, and food texture processing in the brain, and the control of food intake. Physiology & Behavior, **85**(1), 45-56.

169. SAETAE D. & SUNTORNSUK K., 2010. Variation of phorbol esters contents in *Jatropha curcas* from different provenances in Thailand and application of its seed cake as starter broiler diets. American-Eurasian Journal of Agricultural and Environmental Science, **8**(5), 497-501.

170. SAETAE D. & SUNTORNSUK W., 2011. Toxic compound, anti-nutritional factors and functional properties of protein isolated from detoxified *Jatropha curcas* seed cake. International Journal of Molecular Sciences, **12**(1), 66-77.

171. SAETAE D., KLEEKAYAI T., JAYASENA V. et SUNTORNSUK W., 2011. Functional properties of protein isolate obtained from physic nut (*Jatropha curcas* L.) seed cake. Food Science and Biotechnology, **20**(1), 29-37.

172. SAHA S. & GOSH K., 2013. Evaluation of nutritive value of raw and fermented de-oiled physic nut, *Jatropha curcas* seed meal in the formulated diets for Rohu, *Labeo rohita* (Hamilton) fingerlings. Proceesings of the Zoological Society, **66**(1), 41-50.

173. SAMBA S.A.N., DIALLO B., DIOP M., DIATTA M. et SARR A.S., 2007. *Jatropha curcas*: seed germination and propagation methods. Institut Sénégalais de Recherches Agricoles (ISRA)-Laboratoire National des Recherches sur les Productions Végétales (LNRPV). Route des Hydrocarbures, Bel-Air, BP, **3120**, 134-224.

174. SANUSI G.O., BELEWU M.A. et ODUGUWA B.O., 2013. Dietary effects of solid-state fermented *Jatropha curcas* kernel cake on West African dwart goats in a mixed ration. The Pacific Journal of Science and Technology, **14**(2), 448-455.

175. SCHMELZER G.H. & GURIB-FAKIM A., 2008. Plantes médicinales. Ressources végétales de l'Afrique, 11(1), Fondation PROTA/Backhuys Publishers – CTA, Waganingen, Pays-Bas, 870 p.

176. SEBASTIAN S., TOUCHBURN S.P. et CHAVEZ E.R., 1998. Implications of phytic acid and supplemental microbial phytas in poultry nutrition: a review. World's Poultry Science Journal, **54**(1), 27-47.

177. SELANON O., SAETAE D. et SUNTORNSUK W., 2014. Utilization of *Jatropha curcas* seed as a plant growth. Biocatalysis and Agricultural Biotechnology, **3**(4), 114-120.

178. SELJE-ASSMANN N., MAKKAR H.P.S., HOFFMANN E.M., FRANCIS G. et BECKER K., 2007. Quantitative and qualitative analyses of seed storage proteins from toxic ad non-toxic varieties of *Jatropha curcas* L. *In*: Energy and protein metabolism and nutrition, EAAP Publication, 124, Wageningen, 625-626.

179. SELLE P.H. & RAVINDRAN V., 2007. Microbial phytase in poultry nutrition. Animal Feed Science and Technology, **135**(1), 1-41.

180. SHARATH S., MOHANKUMAR B.V. et SOMASHEKAR D., 2014. Bio-detoxification of phorbol esters and other anti-nutrients of *Jatropha curcas* seed cake by fungal cultures using solid-state fermentation. Applied Biochemistry and Biotechnology, **172**(5), 2747-2757.

181. SHARKEY N.A. & BLUMBERG P.M., 1985. Highly lipophilic phorbol esters as inhibitors of specific [$^{3}$H] phorbol 12, 13-dibutyrate binding. Cancer Research, **45**(1), 19-24.

182. SIDDHURAJU P., MAKKAR H.P.S. et BECKER K., 2002. The effect of ionising radiation on aninutritional factors and the nutritional value of plant materials with reference to human and animal food. Food Chemistry, **78**(2), 187-205.

183. SIRISHA P., KUMAR A.A., ANJANEYULU Y. et MADHURI D., 2009. Pathological changes in jatropha (*Jatropha curcas*) deoiled seed cake induced toxicity in broiler chicken and its amelioration. Indian Journal of Veterinary Pathology, **33**(1), 25-29.

184. SIRISHA P., KUMAR A.A., PADMAJA B. et LAKSHMAN M., 2008. Haematobiochemical changes in jatropha deoiled seed cake (*Jatropha curcas*) induced toxicity in broiler chicken and their amelioration. Indian Journal of Veterinary Science, **32**(1), 47-51.

185. SUDAKE K.S., PARNERKAR S., SHANKHPAL S.S., BORANIYA V. et KATOLE S.B., 2013. Feed intake, digetibility, rumen fermentation pattern and blood biochemical profile of growing crossbred calves fed lime treated jatropha (*Jatropha curcas*) cake. Livestock Research International, **1**(1), 8-17.

186. SUMIATI Y.Y., ASTUTI D.A. et SUHARTI S., 2009. Feeding fermented *Jatropha curcas* L. meal supplemented with cellulose and phytase to Kampong chicken. *In* Proceeding, The 1st International Seminar on Animal Industry, Faculty of Animal Science, Bogor Agricultural University, Bogor, 23-24.

187. SUMIATI Y.Y., FARHANUDDIN, SUDARMAN W., ISTICHOMAH N. et SETIYONO A., 2011. Broiler performances fed diet contained *Jatropha curcas* L. meal fermented with *Rhizopus oligosporus*. Media Peternakan, **34**(2), 117-125.

188. SUMIATI Y.Y., SUDARMAN A., NURHIKMAWATI L. et NURBAETI, 2010. Detoxification of *Jatropha curcas* meal as poultry feed. Proceeding of the 2nd International Symposium on Food Security, Agricultural Development & Environmental Conservation in Southeast and East Asia. Bogor, 4-6th September 2007. Faculty of Forestry, Bogor Agricultural University.

189. TENDONKENG F., BOUKILA B., BEDUIDE A. et PAMO T.E., 2009. Essai de substitution du tourteau de soja par la farine de feuilles de *Moringa oleifera* dans la ration finition des poulets de chair. Revue Africaine de Santé et de Productions Animales, **7**(S), 47-52.

190. TERREN M., SAVERYS S., DE HAVESKERCKE P.J., TOUSSAINT A., BAUDOIN P., LOCHT F. et MERGEAI G., 2012. Study of agronomic constraints to the dissemination of the cultivation of *Jatropha curcas* L. in Senegal, Communications in Agricultural and Applied Biological Sciences, **77**, 245-249.

191. TRAORE E.H., 2014. Secteur avicole Sénégal. Revues nationales de l'élevage de la division de la production et de la santé animales de la FAO, **7**, Rome, 70p.

192. UGBOGU A.E., AKUBUGWO E.I., UHEGBU F.O., CHINYERE C.G., UGBOGU O.C. et ODUSE K.A., 2013. Nutritional and chemical composition of *Jatropha curcas* (L) seed from Nigeria. International Journal of Biosciences, **3**(5), 125-134.

193. VAITILINGOM G., 2007. Extraction, conditionnement et utilisation des huiles végétales pures carburant. Enjeux et Perspectives des Biocarburants pour l'Afrique, Ouagadougou.

194. VATS P. & BANERJEE U.C., 2002. Studies on the production of phytase by newly isolated strain of *Aspergillus niger* var Teigham obtained from rotten wood-logs. Process Biochemistry, **30**(2), 211-217.

195. VEERABHADRAPPA M.B., SHIVAKUMAR S.B. et DEVAPPA S., 2014. Solid-state fermentation of jatropha seed cake for optimization of lipase, protease and detoxification of anti-nutrients in jatropha seed cake using *Aspergillus versicolor* CJS-98. Journal of Bioscience and Bioengineering, **117**(2), 208-214.

196. VYAS D.K. & SINGH R.N., 2007. Feasibility study of jatropha seed husk as an open core gasifier feedstock. Renewable Energy, **32**(3), 512-517.

197. WANG H., CHEN Y., ZHAO Y., LIU H., LIU J., MAKKAR H.P.S. et BECKER K., 2011. Effects of replacing soybean meal by detoxified *Jatropha curcas* kernel meal in the diet of growing pigs on their growth, serum biochemical parameters and visceral organs. Animal Feed Science and Technology, **170**(1-2), 141-146.

198. WIDIYASTUTI T., PRAYITNO C.H. et IRIYANTI N., 2013. Digestibility and blood metabolite profiles of chicken fed fermented jatropha seed meal. Animal Production, **15**(2), 98-105.

199. WINA E., TANGENDJAJA B., PASARIBU T. et PURWADARIA T., 2010. Broiler performance fed *Jatropha curcas* seed meal detoxified by fermentattion, physic and chemical treatments. Indonesian Journal of Animal and Veterinary Sciences, **15**(3), 174-181.

200. XIAO J., ZHANG H., NIU L., WANG X. et LU X., 2011. Evaluation of detoxification methods on toxic and antinutritional composition and nutritional quality of proteins in *Jatropha curcas* meal. Journal of Agriculture and Food Chemistry, **59**(8), 4040-4044.

201. ZHANG G., KAZANIETZ M.G., BLUMBERG P.M. et HURLEY J.H., 1995. Crystal structure of the Cys2 activator-binding domain of protein kinase Cδ in complex with phorbol ester. Cell, **81**(6), 917-924.