UNIVERSIDADE DE SÃO PAULO
CENTRO DE ENERGIA NUCLEAR NA AGRICULTURA

TIAGO DO PRADO PAIM

Effect of feeding lambs with cottonseed co-products on reproductive system and meat quality

Piracicaba
2012

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Efeito da alimentação de cordeiros com co-produtos do algodão no sistema reprodutivo e na qualidade de carne

Dissertação apresentada ao Centro de Energia Nuclear na Agricultura da Universidade de São Paulo para obtenção do título de Mestre em Ciências

Área de concentração: Biologia na Agricultura e no Ambiente

Orientador: Prof. Dr. Helder Louvandini

Piracicaba

2012

Dados Internacionais de Catalogação na Publicação (CIP)

Seção Técnica de Biblioteca - CENA/USP

Paim, Tiago do Prado

Efeito da alimentação de cordeiros com co-produtos do algodão no sistema reprodutivo e na qualidade de carne / Tiago do Prado Paim; orientador Helder Louvandini. - - Piracicaba, 2012.

92 p.: il.

Dissertação (Mestrado – Programa de Pós-Graduação em Ciências. Área de Concentração: Biologia na Agricultura e no Ambiente) – Centro de Energia Nuclear na Agricultura da Universidade de São Paulo.

1. Ácidos graxos 2. Metabolismo animal 3. Microscopia eletrônica 4. Ovinocultura 5. Reprodução animal 6. Subprodutos para animais 7. Testosterona I. Título

CDU 636.087 : (636.082 + 613.2)

Dedico este trabalho aos meus pais, Eduardo e Clarice, que me proporcionaram a melhor educação possível, não somente a formal, mas, principalmente, a formação moral, ética, dos princípios e valores.

ACKNOWLEDGEMENTS

Agradeço a Deus por me proporcionar a vida e a saúde, que me permitem seguir esse caminho!

Agradeço aos meus pais, Eduardo e Clarice, por me apoiar incondicionalmente e proporcionar todo o suporte necessário para realização desse trabalho. Agradeço enormemente o quanto se dedicam a mim. Amo vocês!

Agradeço a Taciana, minha irmã, pois sei que estará sempre ao meu lado para o que eu precisar. Sempre estarei com você também. Muito obrigado!

Agradeço a toda minha família, avós, tios e primos. Sei que sempre estão torcendo por mim. Muito obrigado!

Agradeço a Bárbara Borges por ser minha grande companheira, meu apoio, minha conselheira, minha parceira. Você foi muito importante durante esses anos. Com certeza o trabalho seria bem mais árduo sem a sua companhia. Muito obrigado por tudo!

Agradeço a Paulo Borges (*in memorian*) que, além do seu grande incentivo para que eu trilhasse esse caminho, me deixou, sobretudo um grande exemplo.

E agradeço a toda família Oliveira Borges, Márcia, Paula, Carol e Vinícius, pois o apoio de vocês foi muito importante. Muito obrigado!

Agradeço a Aline Campeche pela parceria e apoio mútuo que estabelecemos nesses dois anos. Você foi muito importante durante todo esse caminho. Muito Obrigado!

Agradeço ao Prof. Helder Louvandini pela confiança que sempre depositou em mim e por ter me proporcionar a oportunidade de realizar esse trabalho. Você é parte responsável pela minha formação de Médico Veterinário e, agora, Mestre. Muito Obrigado!

Agradeço a Prof. Connie por toda a orientação e todo o suporte. Desculpe pelos incômodos de vez em quando, inúmeros e-mails, aulas de inglês, projetos... Acima de tudo devo agradecer por ser minha inspiração para carreira acadêmica. Muito Obrigado!

Agradeço ao Prof. Dr. Adibe Luiz Abdalla pela acolhida no laboratório, pelo apoio e incentivo. Muito Obrigado!

Agradeço a Profa. Dra. Carolina Madeira Lucci pela disponibilização do material necessário para realizar o trabalho e pela orientação quanto aos procedimentos relacionados à análise reprodutiva dos animais. Muito Obrigado!

Agradeço a toda equipe NAP/MEPA por me proporcionar a possibilidade de realizar o trabalho de microscopia eletrônica de transmissão. Em especial, agradeço ao Prof. Dr. Francisco A. O. Tanaka por me receber tão bem no laboratório e pelo desprendimento de tentar me ajudar em um assunto tão distante da sua área de trabalho. Muito Obrigado!

Agradeço a Regina Peçanha, Lécio Castilho e Everaldo dos Santos por tudo que aprendi com vocês nesse período e pelo apoio que me proporcionaram. Muito Obrigado!

Agradeço a Pauline Viana, Samara Amador, Eduardo Brandão, Tatiana Barbosa e Caio Cardoso pela enorme contribuição e dedicação durante o período experimental com os animais. Muito Obrigado!

Agradeço aos colegas de trabalho no Laboratório de Nutrição Animal (LANA/CENA): Amr Salah Morsy, Yosra Ahmed Soltan Abd El-Rahman, Ronaldo Lucas, Hani Mohamed Elzaiat, Adibe Luiz Abdalla Filho, Lília Raquel Fé, Edvania Pontes, Patrícia Pimentel, Bernardo Berenchtein, Alessandra Romero, Vitor Guerrini, Laura Cleto, Jade Soares, Taís Cavallaro, Ingrid Mariano, Juliano Issakowicz, Ana Claudia Koki, Andressa Natel, Erika Breda Canova, Gisele Fagundes, Paulo Tavares, Carina Nazato, Fabiana Garcia e Fernanda Campos. Obrigado pelo apoio, conversas, discussões, festas e churrascos!

Agradeço ao CNPq/MAPA (edital 64) pelo suporte financeiro ao projeto e agradeço a FAPESP pela bolsa de mestrado, sem os quais seria impossível a realização do presente trabalho.

Agradeço a Comissão de Pós-graduação (CPG) do Centro de Energia Nuclear pela oportunidade e apoio. E agradeço a toda equipe da secretaria de pós-graduação pelo apoio e dedicação, sempre me atendendo prontamente. Muito Obrigado!

Agradeço ao Centro de Energia Nuclear na Agricultura e a Universidade de São Paulo pela oportunidade de participar do programa de pós-graduação. Muito Obrigado!

ABSTRACT

PAIM, T.P. **Effect of feeding lambs with cottonseed co-products on reproductive system and meat quality.** 2012. 92 f. Dissertação (Mestrado) – Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2012.

The aim of this study was to investigate the effect of feeding cotton co-products with different gossypol concentrations on reproductive development of lambs close to puberty and also on carcass traits and fatty acid profile of meat. Twenty four 5-months old ram lambs (20.6 ± 1.9 kg BW) were used. These were housed in individual pens and received four diets: 20% of dry matter intake (DMI) of whole cottonseed (WCS), 20%DMI of cottonseed meal (CSM), 20%DMI of high oil cottonseed meal (CSC) and a control group without cottonseed co-products (CTL). Free gossypol intake was 0, 16.32, 6.98 and 5.47 mg/kgBW for CTL, WCS, CSC and CSM, respectively. At each 15 days, the animals were weighted, and blood and semen samples were collected. Sperm motility, vigor, mass movement, concentration and pathologies were evaluated. The free testosterone and cortisol concentrations in serum were determined. After 95 experimental days, the lambs were slaughtered and carcass traits were measured. Meat samples of *Longissumus dorsi* muscle were taken for fatty acid profile analysis. And testis samples were collected to analysis in light and transmission electron microscopes. The treatments did not differ in average daily weight gain, sperm volume, motility, vigor and concentration. The CTL group had higher testosterone concentration than CSC at the end of trial and had lower total sperm defects and higher mass movement than others. The number of mitochondrial sheath aplasia increased with increasing gossypol level in diet. There was no relation between the other variables evaluated and gossypol level in diets; however the groups that received cottonseed co-products showed worse reproductive parameters than CTL. Therefore, these co-products had negative impact on reproductive system of puberal lambs. In relation to fatty acid profile, meat from CSM and CSC groups had higher levels of conjugated linolenic acid (CLA) than others and yet CSC group showed higher vaccenic acid than others. Meat from animals that received whole cottonseed had less unsaturated fatty acids, CLA and vaccenic acid. Therefore, between cotton co-products, the processed (CSM and CSC) must be preferred for use in ruminant feed rather than whole cottonseed. The meat from animals that did not receive cotton co-products had higher n-3 fatty acid, and also better n-6 to n-3 ratio compared to others. This can impair the use of these co-products due to current great importance given to these fatty acids in human nutrition.

Keywords: Fatty acids. Whole cottonseed. Infertility. Gossypol. Male.

RESUMO

PAIM, T. P. **Efeito da alimentação de cordeiros com co-produtos do algodão no sistema reprodutivo e na qualidade de carne.** 2012. 92 f. Dissertação (Mestrado) – Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2012.

Com este estudo, objetivou-se avaliar o efeito da alimentação com co-produtos do algodão tendo diferentes concentrações de gossipol no desenvolvimento reprodutivo de cordeiros no período próximo a puberdade e também nas características de carcaça e no perfil de ácidos graxos da carne. Vinte e quatro cordeiros com 5 meses de idade (20.6 ± 1.9 kg PV) foram utilizados. Estes foram alojados em baias individuais e receberam quatro dietas: 20% da ingestão de matéria seca (IMS) de caroço de algodão (CAROÇO), 20%IMS de farelo de algodão (FARELO), 20%IMS de torta de algodão (TORTA) e um grupo controle (CONTROLE) sem o uso de co-produtos do algodão. A ingestão de gossipol livre foi de 0, 16,32, 6,98 e 5,47 mg/kgPV para CONTROLE, CAROÇO, TORTA e FARELO, respectivamente. A cada 15 dias, os animais foram pesados, e amostras de sangue e sêmen foram coletadas. O sêmen foi analisado quanto a motilidade, vigor, turbilhão, concentração e patologias. Foram determinadas a concentração de testosterona livre e cortisol no soro sanguíneo. Após 95 dias de experimento, os cordeiros foram abatidos e as características de rendimento de carcaça e composição da 12ª costela foram mensuradas. Amostras do músculo *Longissumus dorsi* foram coletadas para a análise do perfil de ácidos graxos. Amostras dos testículos foram coletadas para análise em microscopia de luz e microscopia eletrônica de transmissão. Os tratamentos não diferiram em ganho em peso diário, volume ejaculado, motilidade, vigor e concentração espermática. Os animais do grupo CONTROLE apresentaram concentração de testosterona maior que os do grupo TORTA no final do período experimental. E ainda os animais do grupo CONTROLE tiveram menor número de defeitos totais e maior turbilhão que os outros. O número de lesões de aplasia da bainha mitocondrial aumentou com o aumento do teor de gossipol livre na dieta. Para as outras variáveis avaliadas, não houve relação com o nível de gossipol livre na dieta, no entanto os animais que receberam co-produtos do algodão apresentaram piores parâmetros reprodutivos do que os animais do grupo CONTROLE. Por isso, pode-se concluir que estes co-produtos tiveram um impacto negativo no sistema reprodutivo dos cordeiros durante a puberdade. Em relação ao perfil de ácidos graxos, a carne dos animais dos grupos TORTA e FARELO apresentaram maiores valores de ácido linolênico conjugado (CLA) que os outros dois tratamentos e ainda os animais do grupo TORTA apresentaram maior teor de ácido vacênico que os outros. A carne dos animais que receberam caroço de algodão mostrou menor quantidade de ácidos graxos insaturados, CLA e ácido vacênico. Portanto, dentre os co-produtos do algodão, os processados (FARELO e TORTA) devem ser preferidos para uso na nutrição de ruminantes em detrimento ao caroço. A carne dos animais do grupo CONTROLE apresentou maior teor de n-3, e ainda melhor proporção entre n-6 e n-3 comparado com as demais dietas, o que pode dificultar o uso desses co-produtos devido a grande importância dada atualmente a este grupo de ácidos graxos na nutrição humana.

Palavras-chave: Ácidos graxos. Caroço de algodão. Infertilidade. Gossipol. Macho.

ABBREVIATIONS

BW	Body Weight
DMI	Dry Matter Intake
WCS	Whole Cottonseed
CSM	Cottonseed Meal
CSC	Cottonseed Cake
CTL	Control group
GP	Gossypol
FG	Free Gossypol
CP	Crude Protein
EE	Ether Extract
NDF	Neutral Detergent Fiber
ADF	Acid Detergent Fiber
TDN	Total Digestible Nutrients
NEL	Net Energy for Lactation
HPLC	High Performance Liquid Chromatography
TEM	Transmission Electron Microscopy
SAMS	Segmental aplasia of mitochondrial sperm sheath observed in semen samples using TEM
SAMT	Segmental aplasia of mitochondrial sperm sheath observed in testis samples using TEM
SIS	Spermatozoa score (1 to 10) according to number of abnormalities found using TEM
n-3	Group of polyunsaturated fatty acids with a double bond at the third carbon atom counted from the methyl end
n-6	Group of polyunsaturated fatty acids with a double bond at the sixth carbon atom counted from the methyl end
CLA	Conjugated Linolenic Acid
C18:2 c9t11	C18:2 *cis*-9 *trans*-11 – CLA isomer
DHA	Docosahexaenoic Acid – C22:6 n-3
C12:0	Lauric acid
C14:0	Myristic acid

C16:0	Palmitic acid
C18:1 c9	Oleic acid – C18:1 *cis*-9
C18:1t10-t11-t12	*Trans* octadecenoic fatty acids
C18:1 t11	Vaccenic acid – C18:1 *trans*-11
C18:2 n-6	Linoleic acid
C18:3 n-3	Linolenic acid
C20:4	Arachidonic acid
SFA	Saturated Fatty Acids
UFA	Unsaturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
MUFA	Monounsaturated Fatty Acids
DFA	Desirable Fatty Acids [DFA = MUFA + PUFA + stearic acid (C18:0)], according to Landim et al. (2011).
ATHERO	Atherogenicity index (ULBRICHT; SOUTHGATE, 1991)
SBW	Shrunk Body Weight (kg)
HCW	Hot Carcass Weight (kg)
CCW	Cold Carcass Weight (kg)
Y	Carcass Yield (Y=HCW/SBW) (%)
D9C16	Δ^9-desaturase Cis-9 C16 activity
D9C18	Δ^9-desaturase Cis-9 C18 activity
ELONGASE	Elongase enzyme activity

SUMMARY

1. INTRODUCTION

Brazil plays an important role in world cotton market, both in production and exportation. Consequently, there is a great availability of cotton-gin co-products, as whole cottonseed (WCS), cottonseed meal (CSM), cake (CSC) and hulls. Usually, these products had low cost which highlights it as alternative nutrient source for animal nutrition.

The whole cottonseed is considered a special nutritional source. It has a high level of protein and fat, being classified as concentrate. At the same time, it has a high amount of effective fiber, which put it close to roughages. Hence, there is studies using it as substitute of protein sources and also as a substitute of roughage sources (ARIELI, 1998). The cottonseed meals (high and low oil), which are co-products from oil extraction, are considered a protein source replacement. And the cottonseed cake (meal with high oil) also is highlighted by the high fat level (close to 5%).

A great limitation to use of these products is the high gossipol (GP) content. This compound is toxic for animals, causing: appetite loss, respiratory depression, anemia, pulmonary edemas, liver hypertrophy, necrosis of cardiac muscle and sudden death, being well disclosed only the effects in male reproductive system (ROGERS; POORE; PASCHAL, 2002). Generally, the whole cottonseed has higher GP levels than cake which has more than meal. However, the delivery way of GP to animal differ from whole (inside seed) to the other feedstuffs (directly exposed to rumen microbiota), which may impact the biological action of this molecule (ARIELI, 1998).

The main sperm lesions observed in light microscopy evaluation are: isolated head, irregular, notch, broken and/or folded midpiece. The segmental aplasia of mitochondrial sheath is considered pathognomonic of sperm toxicity induced by gossypol (CHENOWETH et al., 2000). The gossypol seems to have a toxic effect in seminiferous epithelium and the subsequent effects are secondary to the initial pathologies (OKO; HRUDKA, 1982a; CHENOWETH et al., 1994). Therefore, one hypothesis is that midpiece abnormalities caused by gossypol in testis led to others sperm pathologies, suggesting that the structural weakness induced during spermatogenesis led to secondary alterations after testis release and, mainly, when the spermatozoa initiates the motility movements.

However, there are great controversial results about the reproductive effects of gossypol, especially in young animals, since few studies evaluating the effect of it in animals at puberal phase are found. Beyond, studies with sheep specifically are very scarce. Hence,

this study aims to evaluate the effect of these cotton co-products in reproductive parameters of lambs during the puberal phase.

Moreover, these cotton co-products have a high fat level, mainly the whole cottonseed. And it is a other factor that limits their wide use, because high fat in ruminant diets (usually above 7%) are related to lower fiber digestibility and consequently lower intake (NRC, 2007). On the other hand, this feature can be beneficial due an increase in energy density of diet and also increase the unsaturated fatty acids accumulated in meat and fat. And, looking for the current search for foods with high levels of these fatty acids (AFMAN; MULLER, 2012), this may proportionate a higher aggregated value for this meat.

Odour and flavour changes in meat attributed to animals fed with whole cottonseed has undergone great discussion in the Brazilian meat industry with the complaints referring to a liver-like flavor (CALKINS; HODGEN, 2007). The medium and long chain unsaturated fatty acids can be related to creating this liver-like off-flavor and, in regression models, the unsaturated fatty acids alone accounted for 40% of the variation in this off-flavor (CALKINS; HODGEN, 2007). Therefore, the evaluation of fatty acid profile changes induced by this feedstuff ant their relations with organoleptic alterations are necessary.

In relation to fatty acid profile and the protein muscle metabolism, the gossypol may also plays a role, since this compound react easily with amines radicals, which is presented in proteins. Therefore, the gossypol can react with a wide range of enzymes, decreasing their activities (CHEN et al., 2009) and affect the protein and lipid composition of muscle, which is a final result of metabolic apparatus of muscle cell. Hence, the present study evaluates the modifications in fatty acid profile of meat from lambs fed with cotton co-products.

1.1. Hypotheses

The negative effect of feeding cotton co-products on reproductive system is related to free gossypol concentration in diet and to its delivery way, inside whole cottonseed or in meals. Feeding cottonseed co-products induce fatty acid profile changes due to high oil level and its delivery form. And gossypol level in diet is related to modifications on fatty acid profile of meat.

1.2. Objectives

This work aims to evaluate the effect of whole cottonseed and cottonseed meals (low and high oil) fed to lambs on reproductive system and meat quality through the evaluation of the follow parameters:

- Scrotal size (circumference, length and width) and seminal parameters
- Sperm and testis structure evaluation using light and electron transmission microscopy
- Male steroids hormones production
- Average daily body weight gain
- Carcass weight and yield at slaughter
- Chemical and centesimal composition of 12^{th} rib and meat.
- Fatty acid profile of meat

2. INTRODUÇÃO

O Brasil possui importante papel no mercado mundial de algodão, tanto em termos de produção quanto de exportação (BRASIL, 2012). Isto gera uma grande disponibilidade de co-produtos originados no processamento do algodão, como caroço, farelo, torta e casca. Geralmente, estes produtos apresentam baixo custo o que os destaca como fonte alternativa de nutrientes para alimentação animal.

O caroço de algodão é considerado uma fonte nutricional diferenciada, pois possui elevado teor de proteína e gordura, o que o classifica como um ingrediente concentrado. E ao mesmo tempo possui uma alta quantidade de fibra efetiva, o que o aproxima dos alimentos volumosos. Dessa forma, existem trabalhos utilizando este como um substituto de fontes proteicas e também como um substituto de fontes de volumoso (ARIELI, 1998). A torta e o farelo de algodão, que são co-produtos do processo de extração do óleo, são considerados substitutos de fontes proteicas. Sendo que a torta, geralmente, também se destaca por apresentar um alto nível de gordura (próximo a 5%).

No entanto, uma grande limitação ao uso desses produtos, é a presença de quantidades elevadas de gossipol. Esta substância é tóxica para os animais, podendo causar perda de apetite, depressão da atividade respiratória, anemia, edemas pulmonares, hipertrofia do fígado, necrose muscular cardíaca e morte súbita, sendo que são bastante divulgados os seus efeitos deletérios no sistema reprodutivo do macho (ROGERS; POORE; PASCHAL, 2002). Geralmente, o caroço de algodão possui maior concentração de GP do que a torta e esta possui mais GP que o farelo. No entanto, a disponibilização do gossipol para o animal difere entre o caroço (dentro da semente) para os outros alimentos (diretamente exposto a microbiota ruminal), e isto pode afetar a ação biológica desta molécula (ARIELI, 1998).

Entre as principais lesões observadas na avaliação por microscopia de luz estão: cabeça isolada e peça intermediária quebrada, dobrada, irregular ou desnuda. A lesão de aplasia segmentar da bainha mitocondrial é considerada patognomônica da toxicidade espermática pelo gossipol (CHENOWETH et al., 2000). Dessa forma, o gossipol parece exercer um efeito tóxico no epitélio seminífero e os efeitos posteriores são, na verdade, secundários as patologias iniciais (OKO; HRUDKA, 1982a; CHENOWETH et al., 1994). Portanto, uma hipótese é de que as anormalidades de peça intermediária causada pelo gossipol no testículo provocam as demais patologias espermáticas, sugerindo que a fraqueza estrutural induzida durante a espermatogênese leva a alterações secundárias depois da saída dos espermatozoides do testículo, e, principalmente, quando estes passam a ter motilidade.

No entanto, tem-se grande controvérsia nos resultados desses efeitos reprodutivos, principalmente em animais jovens, já que poucos estudos avaliando o efeito deste sobre animais durante a fase da puberdade são encontrados. Além disso, especialmente para ovinos existem poucos estudos neste tópico. Por isso, com este estudo, se visa avaliar o efeito desses co-produtos nos parâmetros reprodutivos de cordeiros durante o período da puberdade.

Além disso, esses co-produtos possuem elevado teor de gordura, principalmente o caroço de algodão. E este é outro fator que limita o uso desses na nutrição de ruminantes, já que alto nível de extrato etéreo na dieta de ruminantes (geralmente acima de 7%) está relacionado com menor digestibilidade da fibra e consequente menor consumo (NRC, 2007). Por outro lado, esta pode ser uma característica benéfica destes produtos, já que pode proporcionar aumento da densidade energética da dieta e ainda maior acúmulo de ácidos graxos poliinsaturados na carne e gordura dos animais. E, tendo em vista a atual busca por alimentos ricos neste tipo de ácido graxo (AFMAN; MULLER, 2012), isto poderia proporcionar um maior valor agregado a esta carne.

Alteração de odor e sabor na carne atribuída a animais alimentados com caroço de algodão tem sido um tópico de discussão na indústria brasileira de carne, sendo que as reclamações se referem a um sabor similar a fígado (CALKINS; HODGEN, 2007). Os ácidos graxos insaturados de cadeia média e longa podem estar relacionados na criação desta alteração e, modelos de regressão, apontaram os ácidos graxos insaturados como responsáveis por 40% na variação desse “off-flavor” (CALKINS; HODGEN, 2007). Portanto, a avaliação das alterações no perfil de ácidos graxos na carne causadas por este alimento é necessária para verificar a possibilidade de estarem relacionados com alterações organolépticas.

Em relação ao perfil de ácidos graxos e ao metabolismo proteico muscular, também se pode ter um efeito do gossipol, uma vez que este composto reage muito facilmente com radicais aminos presentes em proteínas. Portanto, o gossipol pode reagir com uma diversa gama de enzimas e transportadores celulares diminuindo a atividade destes (CHEN et al., 2009) e, então, afetar a composição proteica e lipídica do músculo que, em suma, é resultado final do aparato metabólico da célula muscular. Dessa forma, no presente estudo buscou-se avaliar o efeito dos diferentes co-produtos do algodão na dieta dos ovinos sobre o perfil de ácidos graxos na carne.

2.1. Hipóteses

O efeito negativo no sistema reprodutivo da alimentação com co-produtos de algodão está relacionado com a concentração de gossipol livre na dieta e com a forma de apresentação deste na dieta, se dentro do caroço de algodão íntegro ou em farelos. O caroço de algodão na alimentação provoca alterações no perfil de ácidos graxos da carne dos cordeiros devido à alta concentração de óleo e a forma de apresentação deste. E a concentração de gossipol na dieta está relacionada com mudança no perfil de ácidos graxos da carne.

2.2. Objetivos

Este trabalho foi realizado com o objetivo de verificar os efeitos do caroço de algodão, farelo de algodão e torta de algodão utilizado na dieta de ovinos sobre o sistema reprodutivo e qualidade de carne por meio da avaliação dos seguintes parâmetros:

- Parâmetros reprodutivos, como: circunferência escrotal, análise seminal (quantitativa e qualitativa)
- Defeitos espermáticos e alterações na estrutura testicular em microscopia de luz e eletrônica de transmissão
- Produção de hormônios esteróides masculinos
- Ganho em peso dos animais
- Peso e rendimento de carcaça ao abate
- Composição centesimal e química da 12ª costela e da carne
- Perfil dos ácidos graxos na carne

3. LITERATURE REVIEW

3.1. Cotton production in Brazil

Brazilian cotton production in 2011/2012 was 1,992,600 t. The main producing states are Mato Grosso (46.9%), Bahia (31.8%) and Goiás (8.2%). And it is estimated that the production will grow at 3.3% per year in the next ten years (BRASIL, 2012). Brazil is an important exporter in the world, since close to 50% of its production is exported. And it is expected that the exportations will grow at 4.8% per year in the next years (BRASIL, 2012).

The cost of cottonseed transport as harvested is much higher than transport only the lint (SANTOS et al., 2009). Hence, usually, the cotton ginning is carried out next to production site. Consequently, there is a great amount of cottonseed available at low cost, mainly in the three higher producing states (BRASIL, 2012). Thus, this cottonseed is used directly to ruminant nutrition or to oil extraction, which is used to human consumption or biodiesel production.

At the same time, these cotton producing states are great livestock producer, since Mato Grosso represents 15.5%, Bahia (3.8%) and Goiás (9.5%) of total number of cattle slaughtered in the country (BRASIL, 2012). Consequently, it makes simple and cheap the use of these co-products to ruminant nutrition, especially the whole cottonseed.

Biodiesel is gradually gaining acceptance in the world as an environmentally friendly alternative diesel fuel (JANAUN; ELLIS, 2010). In Brazil, as required by law, it must have the addition of biodiesel at 5% to petro diesel as from January 2010, which increased consistently the demand for biodiesel in the country. In March 2011 there were 69 biodiesel production plants authorized by the National Petroleum Agency (ANP) to operate in the country, corresponding to a total authorized capacity of 17,415.95 m^3/day. Biodiesel production in Brazil is predominant based on soy oil (81.36%), but also it is shared between bovine fats (13.36%) and cotton oil (4.11%) (PEREIRA et al., 2012). According to these authors, the biodiesel productive chain generates some sub-products, such as glycerin, lecithin, press cake and oilseed meal, which require further close analysis to their use and valorization, in that they are a key factor in enhancing the economic viability of the production of this fuel.

Therefore, the cotton producing sites in Brazil have a great amount of cottonseed available at low cost and the same sites are bigger livestock producer, providing an increase

use of whole cottonseed in feedlots. Moreover, the great biodiesel demand may drive for oil extraction co-products use, which highlights the further use of pressure-extract meal (cake) and solvent-extract meal. So, it is need consistent research results to support the use of these cotton co-products to ruminant nutrition.

3.2. Nutritional characteristics of cotton co-products

The cotton processing involves initially the separation of long fibers from seeds, process named ginning, that results in a lint seed, which have thin and short fibers (SANTOS *et al.*, 2009). The whole cottonseed can be grinded producing the oil which may be used to human consumption or biofuel production, and generating the co-products. The cottonseed meal is obtained when chemical solvents and pressure are used in oil extraction. And the cottonseed cake (high-oil meal) is obtained when only pressure is used in the process.

The whole cottonseed (WCS) has 23% of crude protein (CP), 17.8% of ether extract (EE), 47% of neutral detergent fiber (NDF), 39% of acid detergent fiber (ADF) and 95% of totals digestible nutrients (TDN), on dry matter basis (NRC, 2007). This composition reflects in its high energy content (9.2 MJ of net energy for lactation, NEL). The CP:NEL ratio (1 g CP to 40 kJ NEL) makes WCS a favorable supplement which meets the combined energy and CP requirements for high-producing dairy cows (ARIELI, 1998). Among the oilseeds, the cottonseed is highlighted by high oil, protein and fiber concentrations (ROGÉRIO et al., 2003). Many studies are found in literature demonstrating a good performance of animals fed with it (ROGERS; POORE; PASCHAL, 2002; BERNARDES et al., 2007; MADRUGA et al., 2008).

Whole cottonseed is a special feedstuff for ruminants. It may be defined as a concentrate due to its high fat and protein content. On the other hand, its rumen effective fiber content is similar to that of roughages (ARIELI, 1998). So, the forage substitution by WCS is a target of some studies as, for example, Bernardes et al. (2007) which used WCS in concentrate (13.5%) *ad libitum* to calves and concluded that it was a good substitute for hay as fiber source in calves' diet.

The protein in the cottonseed kernel consists of storage proteins, the two main ones being of 48 and 52 kD (YU et al., 1996). According to Wadhwa, Makkar and Ichhponani (1993), the fractions of the soluble proteins (albumin 340 g/kg and globulin 230 g/kg) are three times higher than the fractions of the insoluble proteins (prolamine 60 g/kg and glutelin 130 g/k). In vivo experiments with cattle (ZINN; PLASCENCIA, 1993; PIRES et al., 1997)

reported mean values for rumen CP degradability of 74%. A similar mean rumen CP degradability in WCS (77%) was found by in situ rumen incubation in 13 studies using sheep, steers and dairy cows (ARIELI, 1998). Therefore, it is important to consider this high rumen degradability of protein when feeding WCS to ruminants, because depending on diet it may cause an excessive ammonia release in rumen, which can increase nitrogen waste.

The high fat content in WCS can impair the fiber digestibility, which is explained by fat detrimental effect on rumen cellulolytic microorganisms. A compilation of rumen fermentation data (ARIELI, 1998) in which WCS at a level of 120 to 250 g/kg was supplemented to sheep, steers and dairy yielded the following quadratic equation:

$$Y = 3.0\ X^2 - 27.5X + 155$$

$$(n = 16,\ r^2 = 0.36,\ P < 0.05)$$

Where Y = response to WCS expressed as percentage change of acetate to propionate (in relation to basal), and X = basal acetate:propionate ratio. Thus, the acetate:propionate ratio is more likely to increase when feeding WCS with a basal acetate:propionate ratio lower than 3 (concentrate diet). When feeding WCS at a basal ratio higher than 3 (forage diet), a reduced acetate:propionate ratio may be expected (ARIELI, 1998).

In lambs, above 24% of dry matter intake (DMI) of WCS showed a negative effect in NDF, ADF and cellulose digestibility, due to negative effect of lipids on fiber degradation (ROGÉRIO et al., 2004). The EE level in diet used in the study was 6.5% when using 35% of DMI of WCS. So, these authors recommended, for feedlot lambs, the inclusion in diet of WCS in 12 to 24% of DMI.

Lambs fed with CSM (17.9% of DMI) demonstrated a reduction in ingestion and digestibility of dry matter. Similar effect was observed in ewe fed with CSC (20% of DMI) and lambs fed with WCS (20% DMI), which also was justified by high fat content in diet (NAGALAKSHMI; SASTRY; PAWDE, 2003)

On the other hand, the high fat content in diet is related to a lower greenhouse gas emission. Some studies showed that a substitution of soybean meal by cottonseed meals proportionate lower methane production (ABDALLA et al., 2008). According to Arieli (1998), direct measures of methane production revels a reduction of 12 to 14% in ewes fed with 25% DMI of WCS.

When WCS was used in dairy cattle diets, respiration rate decreased and body temperature was lower (COPPOCK; LANHAM; HORNER, 1987). When WCS comprised 250 g/kg of the diet, a decrease of 8% in metabolic heat production was observed in sheep fed

twice maintenance (ARIELI, 1994). The low heat increment of WCS makes it potentially valuable to livestock rearing in tropical climates, and also, in facing global warming (COPPOCK; LANHAM; HORNER, 1987). However, other studies in different thermal conditions did not show consistently decrease in metabolic heat production (ARIELI, 1998). Therefore, it appears that WCS feeding are associated with two opposite effects on energy metabolism. WCS fat may reduce metabolic heat production, whereas its high CP degradability is associated with a high cost of urea formation (ARIELI, 1998).

The nutritional value of cottonseed meal (CSM) can change according to addition of hulls, thus there is cottonseed meals with different crude protein levels available commercially, being more usual meals with 30% and 44% of CP. The CSM with 44% of CP has: 1.8% of EE, 23% of NDF and 77% of TDN (NRC, 2007). In Brazil, Valadares Filho et al. (2010) showed that CSM with 38% of CP has 1.87% of EE, 34.92% of NDF and 68.31% of TDN. The relative high crude protein level and the low cost of CSM turns it an option for animal diet formulation. Also, there are some studies demonstrating good performance in ruminants fed with it (KANDYLIS; NIKOKYRIS; DELIGIANNIS, 1999; ROGERS; POORE; PASCHAL, 2002).

The fat content in CSC is greater than CSM, as the process for obtaining cake uses only the pressure to oil extraction. Data of bromatological composition of cottonseed cake are scarce in literature, NRC (2007) described as the follow composition: 46% CP, 5% EE, 18% ADF, 31% NDF and 80% of TDN.

The cottonseed hulls are the external layer of seed with some adhered linter. This product shows a high fiber level, which makes it interesting to use it as alternative roughage feed to ruminants (CHIZZOTTI et al., 2005; MAGALHAES et al., 2005). It is surprising that hulls have good palatability to cattle (ROGERS, POORE; PASCHAL, 2002). The hull composition is highly variable because efficiency of lint extraction is not constant, leading to different proportions between linter and kernel. The average hull composition are 4.2% CP, 2.93% EE, 61.70% FDA, 77.68% FDN and 42% TDN (NRC, 2007).

All these cottonseed products have some degree of remainder cottonseed oil. According to Gioielli (1996), the cottonseed oil is composed by approximately 30% of saturated fatty acids, 50% of linoleic acid (C18:2) and 20% of monounsaturated fatty acid, thus this is a good source of unsaturated fatty acids. So, the high concentration of fatty acids in WCS, and also CSC, must be considered to their use in ruminant nutrition, because it permits to increase the energetic density of diet without decrease the fiber and protein levels.

Moreover, this can incorporate more unsaturated fatty acids in the animal products (milk and meat).

A great limitation to use of these products is the gossypol content. This substance is toxic to animals as it can react with various organic compounds. There are many discussions about the harmful effect of gossypol, mostly in male reproductive system. Thus, some breeders avoid using these products in animal nutrition. Indeed, one main concern is about gossypol accumulation in meat, which already was demonstrated by Kim, Calhoun and Stipanovic (1996) and can lead to organoleptic changes in product, beyond a possible toxic effect on consumers.

3.3. Gossypol

A major limitation to use of cottonseed products in animal nutrition is gossypol ($C_{30}H_{30}O_8$) (Figure 1). This compound is present in members of the *Malvaceae* family such as the cotton plant, being a naturally occurring toxin that deters insect pests. It is a yellow, polyphenolic aldehyde compound, which is present in the highest concentrations in cottonseed pigment glands (TILYABAEV et al., 2009).

This compound exist as a mixture of two stereoisomers (+) and (-), since the negative has higher biological activity (McCAUGHEY et al., 2005). And also, the gossypol is a highly reactive substance that acts as phenolic and aldehydic compound. The phenolic groups react to form esters and ethers, and the aldehyde groups react with amines (proteins) to form Schiff's bases (EL-SHARAKY et al., 2010). Moreover, the gossypol exists in free and bound forms. The bound forms usually occur in higher concentrations than free form, except to WCS which contains more of the free form. The free form has higher biological activity.

The first biological effect of gossypol detected was the male infertility, which highlighted it as a possible male contraceptive drug. Recently, gossypol and its analogs have been studied extensively for their broad-spectrum of biological activities, such as anti-parasitic, anti-malarial, anti-HIV and anticancer (ZHANG et al., 2009).

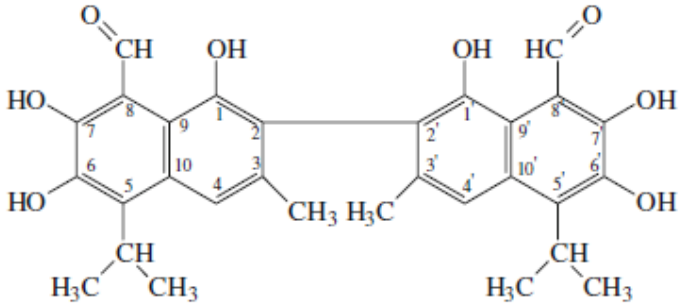

Figure 1 – Gossypol structure (aldehyde tautomer) (TILYABAEV et al., 2009).

3.3.1. Gossypol toxicity

The gossypol may cause some serious problems as: appetite loss, respiratory depression, anemia, pulmonary edema, dyspnea, erythrocyte fragility, liver hypertrophy, necrosis of heart muscle, reproductive problems and sudden death (BULLOCK et al., 2010). The most consistent histological finding in gossypol toxicosis is hepatic centrilobular fatty change and necrosis. Gossypol is also a cardiotoxin, although the mechanism of the cardiac toxicity is unclear. And yet, generalized edema is a common postmortem lesion. Large amounts of straw-colored fluid of high-protein content are consistently found in the thoracic and peritoneal cavities and pericardial sac. Subcutaneous edema has been reported to be prominent throughout the ventral cervical and thoracic region. A comprehensive review about clinical signals of gossypol toxicosis are found in Rogers, Poore and Paschal (2002).

The toxic effect of GP varies according to intake, duration, age of animal and stressor conditions (GAMBOA et al., 2001). Diet composition plays an important role in the development of gossypol toxicity. High-concentrate diets favor gossypol toxicosis because faster passage rate and lower ruminal pH, which allow gossypol to pass through the rumen unbound (DANKE; PANCIERA; TILLMAN, 1965). Apparently, animals can tolerate higher levels of free gossypol (FG) in WCS than in CSM. The gossypol in WCS is possibly released more slowly because more time is spent in the rumen than with CSM. Another reason for this slower release is that some cottonseed remains unbroken after chewing, resulting in a more gradual GP release as cottonseeds are ruminated (ROGERS; POORE; PASCHAL, 2002).

The maximum doses recommendation found in literature are quite different. Arieli (1998) determined that, for bovine males at growing, up to 200 mg/kg of FG was safe, 400 mg/kg was toxic and 800 mg/kg of FG caused some deaths. On the other hand,

Rogers, Poore and Pachal (2002) recommended maximum FG levels in diet equal to 200 mg/kg for pre-ruminants, 900 mg/kg for growing cattle, 600 mg/kg for young bulls, 900 mg/kg for adult bulls and 1200 mg/kg for adult cows.

In study with lambs, a diet with 20 mg/kg of Body Weight(BW)/day of FG did not cause any intoxication signal, indicating that this concentration is safe to use in lamb feedlots (KANDYLIS; NIKOKYRIS; DELIGIANNIS, 1998). And yet, in this same study, the group that received WCS had high average daily gain, better feed conversion and higher carcass yield in relation to control group. On the other hand, it was observed FG accumulation in liver, kidneys and heart in the same lambs (NIKOKYRIS; KANDYLIS; DELIGIANNIS, 1999). And yet, it was observed an increase in lactate dehydrogenase activity and in plasmatic urea concentration, indicating a metabolic response to toxicological effects.

Fthenakis et al. (2004) reported high mastitis prevalence (94% of herd) caused by *Staphylococcus aureus*, in a sheep herd fed with 25% of CSM during two years. The gossypol in diet was indicated as a predisposition factor for disease development. It was found GP in milk from these ewes which prove the GP absorption and the possibility of systemic action. These authors argued that GP has an immunological depressor effect due lipoxigenase inhibition.

Andreazzi et al. (1998) did not find GP in plasma from male caprines that received 30%DMI of WCS (13.45 mg of FG/kgBW/day) during 18 months. These authors concluded that GP was not absorbed, or the amount absorbed was insignificant, possibly due to high capacity of gossypol to form complex with proteins in rumen, which prevents the intestinal absorption.

3.3.2. Factors affecting gossypol concentration

Most gossypol found in WCS is in the free form, but some becomes bound due to the heat, moisture and pressure associated with cottonseed meal processing. The bound form of gossypol is generally considered to be nontoxic to ruminants, although it has been suggested that some bound gossypol from processed WCS or CSM may be converted to free gossypol during digestion (NOFTSGER et al., 2000; MENA et al., 2001).

Robinson et al. (2001), analyzing the GP level in WCS from different varieties of Pima type (*Gossypium barbarense*), found few variation in FG content (1.04% to 1.15% of DM), and the isomers proportion {0.43% DM for (+) and 0.56% DM for (-)}. Nevertheless,

Chenoweth et al. (2000) observed that CSM had a variation of 1.25% to 1.60% of FG, values higher than those found in WCS.

The GP content in cotton can change according to environmental conditions, specie and variety of cotton plant. As the environment temperature of growing site increases, lower the GP content, and as rainfall increases, higher the GP content in plant. In relation to species, the *Gossypium hirsutum*, usually named as Upland, has lower levels than the *Gossypium barbarense*, usually named as Pima (McCAUGHEY et al., 2005).

In plants, the GP act as defense against insects and pests. Varieties without gossypol are already developed; however, these are not adopted in commercial crops, because this variety is susceptible to some pests, which consequently implies in higher costs with pesticides. Romano and Scheffler (2008) suggest a crossbreeding scheme that reduces the gossypol content in seeds and maintains a concentration in vegetative portion of plant that is enough to protection against pests. After seven generations of selection, these authors identified plants that had a reasonable gossypol concentration in critical defense points and showed less than 0.3% of total gossypol in seeds. So, the authors concluded that it is an efficient way of producing cotton and also a safe form to provide protein and energy for animals.

The processing can reduce the FG content because this is a highly reactive compound that easily forms bounds with proteins. The lysine seems to be the main aminoacid that binds to FG, which block its absorption by animal. This is an important feature of processed cotton co-products, mainly for poultry and swine nutrition, because sometimes it leads to reduced aminoacid availability.

The binding of FG in CSM can be obtained by using solvent extraction, cooking, autoclaving, pelleting, as well as adding calcium and iron salts. The cooking at 100 °C during 10 minutes reduced in 44% the FG content (JARQUIN et al., 1966). Zhang et al. (2007) verified that employing heat (130 °C for 20 min) reduced the FG levels. Therefore, the heat treatment of these products is potentially able to reduce significantly the FG content. Nagalakshmi, Sastry and Pawde (2003) concluded that cooking and adding 1% calcium hydroxide ($Ca(OH)_2$) can be used in cotton products aiming to reduce gossypol effects in animals. These same authors did not recommend the iron addition due to its inefficacy.

Microbial fermentation also is pointed as able to reduce FG level in CSM. The *Candida tropicalis* ZD-3 had showed as the most efficient microorganism in gossypol toxicity reduction (ZHANG et al., 2007). At the same study, the supplementation with glucose and

sacharose, as well as the heat treatment improved the bacterial fermentation and consequently the FG detoxification.

3.3.3. Methods for gossypol analysis

The determination of gossypol content with high precision is very important, because gossypol has been the target of a wide range of recent researches with animals and humans (TALIPOV et al., 2009). There are two main methods for gossypol determination, one using high performance liquid chromatography (HPLC) and other using spectrophotometry, which is recommended by American Oil Chemistries Society (AOCS) (HRON; KUK; ABRAHAM, 1990). Both methods depend on extraction and derivation procedures. The derivation consists on promote gossypol binding to others substances to enhance the molecule stability and to permit the quantification, since the best results are found when gossypol is bind to lysine (WANG; PLHAK, 2000).

According to Wang and Plhak (2004), the HPLC and colorimetric methods presents a great variation in results due to its empirical nature and the various types of bond gossypol. The AOCS method is laborious and suffers influence of other terpenoids compounds present in samples from cotton products. The HPLC method demands a very detailed and careful sample preparation. On the other hand, Hron, Kuk and Abraham (1990) state that HPLC is a simple and sensible method, and yet it has reproducible results.

The development of anti-gossypol polyclonal antibodies was reported by Wang and Plhak (2000). However, the sample derivation was still required because the antibodies only recognize the gossypol in conjugated form. So, in Wang and Plhak (2004), the authors presented a monoclonal antibodies method, which has the ability of recognizing directly the gossypol, both bound and free forms, eliminating the requirement of derivation and providing better comprehension of biological function of each form. And yet, this last method demonstrated high correlation with AOCS method.

The great variation in gossypol content in cotton products stands that the recommendation for animal nutrition must be criterions according to gossypol level. However, there is a lack of fast and practical methods to gossypol determination, as well the lack of enabled laboratories for this type of analysis in Brazil (PAIM et al., 2010). The development of accurate methods to gossypol analysis in whole cottonseed is facing some difficulties due the great variation between one seed to another and between samples. And

nowadays, little is being done to development of analysis method for gossypol residues in milk and meat, as well as liver, kidney and heart.

3.3.4. Reproductives effects of gossypol

The GP is widely known as male antifertility agent (GIZEJEWSKI et al., 2008). This compound seems to induce infertility through spermatogenesis suppression, hence this has been evaluated to male human contraception (COUTINHO, 2002). And the main remaining question to its application is about the process reversibility.

The females are considered relatively resistant (ROGERS; POORE; PASCHAL, 2002). The long-term feeding (431 days) whole cottonseed (15% of DMI and 1300 mg/kg of GP), beginning at 3 months of age, did not show deleterious effect in heifers at onset of puberty or pregnancy rate (COLIN-NEGRETE; KIESLING; ROSS, 1996). Nevertheless, these same authors informed that 30% DMI of WCS (2.000 mg/kg of GP) is dangerous due to high erythrocyte fragility.

The reproductive effects of GP in ruminants have controversial results. Negative effect of GP in male bovine reproduction has been well reported (RANDEL; CHASE; WYSE, 1992). Although, according to Rogers, Poore and Paschal (2002), the true impact of the main abnormalities found in gossypol treated animals on male fertility is not well defined.

The segmental aplasia of mitochondrial sheath is considered a pathognomonic sign of sperm toxicity by gossypol (CHENOWETH et al., 2000). The GP causes a toxic effect in seminiferous epithelium and post-testis effects are secondary, possibly due to initial pathologies (OKO; HRUDKA, 1982a; CHENOWETH et al., 1994). Therefore, the hypothesis is that the midpiece abnormalities caused by GP provoke the others sperm pathologies, suggesting that structural weakness induced during spermatogenesis leads to secondary alterations in spermatozoa after testis releasing (OKO; HRUDKA, 1982b).

The GP effects in bovine male are dose and time dependent. Arshami and Ruttle (1988) reported seminiferous tubules with large lumen, lower epithelium thickness and reduced number of cell layers of germinative epithelium in young bovine males fed with 0.69% of FG diet during two months. Then, the same animals received a diet without gossypol and the histological testis recovery was observed. It indicates that GP effects on fertility in young males are reversible, at least partially (ARSHAMI; RUTTLE, 1988). These authors informed that positive effect of diet without gossypol is seen only after six to eight

weeks, which corresponds to interval required to production of mature spermatozoa from primordial cells.

Adult bovine males fed with CSM and WCS (3.2 and 32 g of GP/day, respectively) did not show gossypol effect on scrotal circumference, sperm concentration, motility and morphological abnormalities of spermatozoa (observed in light microscopy) (CHASE et al., 1994). In previous reports, the quality and quantity of sperm from bulls and rams also did not have suffered effect of GP (ARSHAMI, 1989; JIMENEZ; CHANDLER; ADKINSON, 1989). Randel, Chase and Wyse (1992) demonstrated that animals fed with gossypol diets had normal sperm under light microscopy. On the other hand, further detailed analysis found lesions in cell membrane of spermatozoa, which cause deleterious effect in sperm tail.

The gossypol seems to cause higher damage in reproductive function of ruminant males close to puberty than in mature males. Young bovine males were fed from weaning until puberty (196 days) with three diets: WCS with 60 mg/kg of FG; CSM with 6 mg/kg of FG and soybean meal without gossypol. The WCS group showed lower average daily gain and the onset of puberty were later compared to other groups. The puberty was reached at similar body weights between groups, indicating that delayed puberty of bulls fed with WCS may be consequence of the lower performance and not a directly effect of gossypol. Also it has presented no reduction in quality and quantity of sperm in light microscopy (CHASE et al., 1994).

Bulls, 20 months old, fed with 8.2 g of FG/day during 11 weeks demonstrated that normal spermatozoa percentage was lower than control group. The sperm abnormalities detected using contrast phase microscopy involve the midpiece and, consequently, lower sperm motility. The live spermatozoa percentage in gossypol group was not changed, but there was a significant reduction on normal spermatozoa count. This indicated that gossypol has deleterious effects in specific sperm structures, mainly midpiece, without changes in sperm membrane viability (CHENOWETH et al., 1994). On the other hand, Cusack and Perry (1995) concluded that adult bovine males fed with WCS up to 19.8 g of FG/animal/day did not have effect on fertility.

Santos et al. (2008) evaluated the diet with 14%DMI of WCS (2.2 kg/animal/day) given during 73 days to bulls, and observed that this diet reduced the motility and increased the percentage of major defects and total sperm defects. The Sertoli cells showed wrinkled membrane, lipids accumulation in cytoplasm, deformed mitochondria, spermatids apoptosis,

altered nuclear membrane, vacuolated Golgi complex and abnormal chromatin localized in nuclear polo.

Gizejewski et al. (2008), feeding deer with 350g of WCS per day (15 mg of GP/kgBW/day) during 109 days, found morphological abnormalities in spermatozoa, decreased sperm motility and spermatogenesis abnormalities. The steroidal hormones levels (Testosterone, A4-androstenedione and E2-Estradiol-17β) in seminal plasma were lower leading to lower semen quality. In the following year, it was observed a recovery of sperm quality, showing the reversibility of gossypol induced infertility, and these authors concluded that feeding gossypol can be used as efficient contraception method to male deer.

3.4. Fatty acids

3.4.1. Fatty acids in human nutrition

Dietary fatty acids influence human health in numerous ways and influence several indicators of health status. Recent studies revealed that this would be linked to gut-derived endotoxemia during fat digestion in high-fat diets and also related to fatty acid profile of this diet. Contemporary human diet contains excessive quantities of saturated fatty acids (SFA) and n-6 fatty acids, but it is deficient in n-3 fatty acids (AFMAN; MULLER, 2012). This imbalance could be the cause of respiratory diseases, obesity and cancer. According to Laborde et al. (2001), every 1000 kcal consumed should deliver a maximum of 11 g SFA, a minimum of 3300 mg n-6 and a minimum of 500 mg n-3. According to contemporary nutritional standards, a healthy n-6/n-3 ratio is 2-4 to 1, whereas in developed countries, the actual ratio is 10-14 to 1 (SIMOPOULOS, 2001; 2002).

Based on the accumulated results of numerous studies, Crawford (1992) defined a new theory of evolution, which proposes that n-3 and n-6, especially the docosahexaenoic acid (DHA), helped to form the human brain. Thus, these authors concluded that the health of the next generations is very dependent on the assurance of proper nutrition of future mothers during pregnancy, and that n-3 and n-6 in balanced proportions, can play the key role in this process (KOCHMAN, 2012).

Studies evaluating the gene expression changes caused by high n-3 and SFA diets showed that larger part of the changes in the n-3 could be attributed to a down-regulation in expression of immune related genes and genes known to be involved in development of atherosclerosis. Additional changes were related to an increased expression of cell-cycle

genes and genes involved in transcriptional and translational regulation (AFMAN; MULLER, 2012).

In study with mice, the palm oil-based diet resulted in the greatest inflammatory outcomes and, in contrast, a rapeseed oil-based diet seemed to result in a metabolism driven toward less inflammatory pathways. This shows that dietary fat composition can contribute to modulate the onset of low-grade inflammation through the quality of endotoxin receptors (LAUGERETTE et al., 2012).

In recent years much work has focused on elucidating more precisely how dietary fatty acids lead to short-and long-term changes in cellular functions. There is now considerable evidence that fatty acids, in particular unsaturated fatty acids, exert many of their biological effects through modulation of gene transcription by regulating the activity of numerous transcription factors, including sterol regulatory binding proteins (SREBPs) or nuclear receptors such as peroxisome proliferator activated receptors (PPARs) and liver X receptors (LXRs) (AFMAN; MULLER, 2012).

Until recently, animal fat, mainly meat fat, was regarded as a source of saturated fatty acids, which are a risk factor for atherosclerosis, obesity and certain types of cancer. Recent studies have shown, however, that ruminant fats contain biologically active substances beneficial for health, and that only some saturated fatty acids have adverse consequences. The latter group includes lauric acid (C12), myristic acid (C14) and palmitic acid (C16). Ruminant meat also has the most desirable n-6/n-3 ratio (6.3:1) in comparison with pork (12.7:1) and poultry (8.3:1) (MICINSKI et al., 2012).

Conjugated linoleic acid (CLA), in particular its cis-9 trans-11 and trans-9 cis-11 isomers, is one of the substances found in beef with health-supporting properties. This fatty acid is synthesized by rumen microbiota because it is a biohydrogenation intermediary. There are many researches confirming CLA's ability to reduce the risk of atherosclerosis and obesity, and to lower cholesterol levels. CLA prevents and alleviates the symptoms of type 2 diabetes. It is also a powerful antioxidant, and yet it boosts immunity. And, when incorporated into the human diet in the amount of 1.5 to 3.5 g, CLA exerts anticarcinogenic effects (it inhibits the development of breast cancer, malignant melanoma, colorectal cancer and lung cancer) (MICINSKI et al., 2012).

Therefore, the major nutritional problems facing the world today (e.g. obesity, diabetes and malnutrition) require multidisciplinary groups working together in large national and international consortia (AFMAN and MULLER, 2012). Therefore, the linkage between

concerns in human nutrition, animal food composition and animal nutrition is an essential part in it.

3.4.2. Fatty acids in animal nutrition

There are evidences that the feed offered to animals can change the fatty acid profile of meat and milk, which could permitted the manipulation of fat composition (FRENCH, STANTON, LAWLESS, 2000). Still, the use of unsaturated fatty acids in protected form or whole seeds in ruminant feed is interesting because it could improve the nutritional value of food from animal sources affording value aggregation to it (RAES; DE SMET; DEMEYER, 2004).

The use of green forage in the diet of ruminants increases the n-3 content in beef fat, guarantees a healthier n-6/n-3 PUFA ratio and increases the concentrations of non-enzymatic antioxidants: vitamin E, ascorbic acid and beta-carotene. According to Micinski et al. (2012), the incorporation of oilseed plants, plant fats and green forage in cattle's diets is believed to be the most effective method of increasing the concentrations of desirable fatty acids in beef.

As the ratio of unsaturated to saturated fatty acids are 72:28 in WCS (ARIELI, 1998), it stands out as an important unsaturated fatty acids source to be exploited in animal nutrition. It was suggested that WCS fat may leave the rumen still partially enclosed within the seed, and thus promote the incorporation of more polyunsaturated fatty acids in meat and milk (ARIELI, 1998), which is beneficial in terms of human nutrition claims.

However, the manipulation of meat fat requires some caution. High polyunsaturated fatty acid content also is related to odor and flavor changes in meat. And yet, it can lead to shorter shelf life, due to faster oxidation process. Some Brazilian feedlots are refusing to use whole cottonseed as it is pointed out as causing the liver-like flavor complaints, which may be related to changes in meat fat content and profile (CALKINS; HODGEN, 2007).

Studies realized by Coppock and Wilks (1991), evaluating the lipid inclusion in diet of dairy cows, showed that the lipids from oilseeds provide a slowly fat release throughout the day, due to regurgitation and chewing. Supplementing WCS to steers, Zinn and Plascencia (1993) demonstrated an increased saturated and decreased unsaturated fatty acids in the small intestine, indicating extensive hydrogenation of WCS within the rumen. This is good because it decreases the inhibitory effect of fat on fiber digestibility. But, it is a problem since it hinders the incorporation of unsaturated fatty acids in meat and milk.

Oliveira et al. (2011) evaluated the addition of ground soybeans (SB), ground cottonseed (CS) and ground linseed (LS) in diet of finishing steers and observed that the greatest percentages of myristic acid (C14:0), palmitic acid (C16:0), *trans* octadecenoic acid (C18:1 *trans*-10, *trans*-11, or *trans*-12), and SFA in the subcutaneous fat were observed in the CS treatment. Moreover, the lowest percentages of oleic acid (C18:1 *cis*-9) and total unsaturated fatty acids in the subcutaneous fat were observed in the CS diet. The n-6 and n-3 percentages were greatest in the SB and LS treatments, respectively. These authors concluded that the fatty acid profile of subcutaneous fat was impaired by the addition of CS and that supplying ground oilseeds did not increase the content of CLA in the meat.

Kim et al. (2007), studying four n-6:n-3 ratios in lamb diet, found that ruminal digesta from lambs fed the lowest n-6:n-3 ratio contained a greater proportion of C18:0 compared with those fed the greatest n-6:n-3 ratio, indicating that C18:3n-3 underwent greater complete biohydrogenation than C18:2n-6. In some way, n-3 fatty acids are preferred to hydrogenation by rumen microbiota rather than n-6. This conclusion highlighted the difficulty to promote high n-3 incorporation in meat and milk.

Conjugated linoleic acid is also an intermediate in the ruminal biohydrogenation of C18:2n-6 to C18:0. However, Beaulieu, Drackley and Merchen (2002) reported that increased dietary intake of C18:2n-6 did not affect *cis*-9 *trans*-11 CLA concentrations in ruminal contents of steers fed a soybean oil supplement. Kim et al. (2007) also observed a lack of response in *cis*-9 *trans*11 CLA concentration in ruminal digesta due to increase C18:2n-6 content in diet.

Therefore, a great variation in results of fatty acids manipulation is found in literature, some studies pointed an increase in polyunsaturated fatty acids in milk and meat (MADRUGA et al., 2008) and, on the contrary, other authors showed an increase in saturated fatty acids in meat (MAIA; BRANCO; MOURO, 2006) and milk (ARIELI, 1998). Probably, these variable results are related to differences in ruminal environment. As demonstrated to fat effect on rumen-fermentation characteristics, the diet composition can affect at some extent the biohydrogenation process. So, a high concentrate diet generally is related to a higher feed intake, low rumen pH and less rumination, which might explain the relatively lower biohydrogenation and higher rumen bypass observed in some studies (PIRES et al., 1997).

The rumen biohydrogenation hampers fatty acid composition changes of meat and milk. Thus, one way of dealing with it is the fat protection. A method to protect the

polyunsaturated fatty acids (PUFA), mainly the n-3 fatty acids, from rumen biohydrogenation could be important to improve the n-6:n-3 ratio (OLIVEIRA et al., 2012). In a study with dairy cows, heated WCS treatment protected fat from hydrogenation in the rumen, which was proved by the higher C18:2 content in milk fat (PIRES et al., 1997). Moreover, these authors found that roasting WCS increases the content of C18:2 in the duodenal flow proving the protection against rumen microbiota.

Nevertheless, Oliveira et al. (2012) found controversial results when evaluating the effect of protected fat sources in diet of steers. These authors observed more conjugated linoleic acid (CLA) in the meat of animals fed with unprotected soybean oil, while better n-6/n-3 ratio were noted for those fed unprotected linseed oil. Therefore, these results highlight the complexity of rumen biology.

On the other hand, some studies in dairy cows demonstrated that increase unsaturated fatty acid in diet can led to reduction on fat concentration in milk. It happens because intermediate biohydrogenation products, as C18:1 *trans*-11, inhibit fat synthesis in mammary gland (PERFIELD et al., 2007; SARTORI; GUARDIEIRO, 2010).

Still, diets with high polyunsaturated fatty acids can improve the fertility of females. There are studies showing an effect of fatty acids on number and size of ovarian follicles, size of corpus luteum and ovum quality (BELLOWS et al., 2001). And yet, other studies observed that high fat diets improve the embryo quality and increase the steroids hormones and prostaglandin concentrations (WILLARD et al., 1995). Probably, the association between lipids and steroids occurs by increase in cholesterol concentrations, which is a precursor of steroid synthesis.

According to Willard et al. (1995), the supplementation with WCS to beef cows at the end of gestation is an efficient method to improve reproduction success. These authors used pregnant cows supplemented with WCS, at three free gossypol (FG) levels (0, 2 and 4 g/animal/day) from 90 days prepartum until 112 days postpartum. The majority of cows fed with WCS showed luteal activity between 96 and 105 days postpartum. The interval between calving and conception was lower in animals receiving more WCS and consequently the pregnancy rate at 112 days postpartum was higher (66.7% with 2g of FG, 73.3% with 4g of FG versus 33% of control).

REFERENCES

ABDALLA, A. L. et al. Utilização de subprodutos da indústria de biodiesel na alimentação de ruminantes. **Revista Brasileira de Zootecnia**, Viçosa, v. 37, p. 260-268, 2008. ISSN 1516-3598.

AFMAN, L.; MULLER, M. Human nutrigenomics of gene regulation by dietary fatty acids. **Progress in Lipid Research**, Amsterdam, v. 51, n. 1, p. 63-70, 2012. ISSN 0163-7827.

ANDREAZZI, M. A. et al. Determinação de gossipol plasmático em caprinos, utilizando cromatografia líquida de alta pressão (HPLC). **Arquivos de Ciências da Saúde Unipar**, Umuarama, v. 2, n. 3, p. 225-229, 1998.

ARIELI, A. Effect of whole cottonseed on energy partitioning and nitrogen-balance in sheep. **Animal Production**, Cambridge, v. 58, p. 103-108, 1994. ISSN 0003-3561.

_____. Whole cottonseed in dairy cattle feeding: a review. **Animal Feed Science and Technology**, Amsterdam, v. 72, n. 1-2, p. 97-110, 1998. ISSN 0377-8401.

ARSHAMI, J.; RUTTLE, J. L. Effects of diets containing gossypol on spermatogenic tissues of young bulls. **Theriogenology**, New York, v. 30, n. 3, p. 507-16, 1988. ISSN 0093-691X.

ARSHAMI, J.; RUTTLE, J. L. Effects of diets containing cottonseed meal on semen quality and testicular tissue in fine-wool rams. In: WESTERN SECTION, AMERICAN SOCIETY OF ANIMAL SCIENCE, 40., 1989, Laramie, WY. **Proceedings**… Champaign, Il: ASAS, 1989. p. 277-279.

BEAULIEU, A.; DRACKLEY, J.; MERCHEN, N. Concentrations of conjugated linoleic acid (cis-9, trans-11-octadecadienoic acid) are not increased in tissue lipids of cattle fed a hiph-concentrate diet supplemented with soybean oil. **Journal of Animal Science**, Albany, v. 80, n. 3, p. 847-861, 2002. ISSN 0021-8812.

BELLOWS, R. A. et al. Effects of feeding supplemental fat during gestation to first-calf beef heifers. **The Professional Animal Scientist**, Fayetteville, v. 17, n. 2, p. 81-89, 2001. Disponível em: < http://pas.fass.org/content/17/2/81.abstract >.

BERNARDES, E. B. et al. Efeito da substituição do feno de Tifton 85 pelo caroço de algodão como fonte de fibra na dieta de bezerros. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v. 59, p. 955-964, 2007. ISSN 0102-0935.

BILBY, T. et al. Effects of dietary unsaturated fatty acids on oocyte quality and follicular development in lactating dairy cows in summer. **Journal of Dairy Science**, Lancaster, v. 89, n. 10, p. 3891-3903, 2006. ISSN 0022-0302.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Brasil projeções do agronegócio 2011/2012 a 2021/2022**. Brasília, DF, 2012. 50 p.

BULLOCK, S. et al. Plasma gossypol dynamics in white-tailed deer: Implications for whole cottonseed as a supplemental feed. **Small Ruminant Research**, Amsterdam, v. 93, n. 2-3, p. 165-170, 2010. ISSN 0921-4488.

CALKINS, C.; HODGEN, J. A fresh look at meat flavor. **Meat Science**, Barking, v. 77, n. 1, p. 63-80, 2007. ISSN 0309-1740.

CHASE, C. et al. Growth and reproductive development in brahman bulls fed diets containing gossypol. **Journal of Animal Science**, Albany, v. 72, n. 2, p. 445-452, 1994. ISSN 0021-8812.

CHEN, B. et al. The (+)- and (-)-gossypols potently inhibit human and rat 11 beta-hydroxysteroid dehydrogenase type 2. **Journal of Steroid Biochemistry and Molecular Biology**, Amsterdam, v. 113, n. 3-5, p. 177-181, 2009. ISSN 0960-0760.

CHENOWETH, P. et al. Characterization of gossypol-induced sperm abnormalities in bulls. **Theriogenology**, New York, v. 53, n. 5, p. 1193-1203, 2000. ISSN 0093-691X.

_____. Effects of dietary gossypol on aspects of semen quality, sperm morphology and sperm production in young brahman bulls. **Theriogenology**, New York, v. 42, n. 1, p. 1-13, 1994. ISSN 0093-691X.

CHIZZOTTI, M. et al. Partial replacement of elephantgrass silage with cottonseed hulls. 1. Intake, degradability, and apparent ruminal, intestinal, and total tract digestibilities in steers. **Revista Brasileira de Zootecnia**, Viçosa, v. 34, n. 6, p. 2093-2102, 2005. ISSN 1516-3598.

COLIN-NEGRETE, J. et al. Effect of whole cottonseed on serum constituents, fragility of erythrocyte cells, and reproduction of growing Holstein heifers. **Journal of Dairy Science**, Lancaster, v. 79, n. 11, p. 2016-2023, 1996. ISSN 0022-0302.

COPPOCK, C.; LANHAM, J.; HORNER, J. A review of the nutritive-value and utilization of whole cottonseed, cottonseed meal and associated by-products by dairy-cattle. **Animal Feed Science and Technology**, Amsterdam, v. 18, n. 2, p. 89-129, 1987. ISSN 0377-8401.

COPPOCK, C.; WILKS, D. Supplemental fat in high-energy rations for lactating cows - effects on intake, digestion, milk-yield, and composition. **Journal of Animal Science**, Albany, v. 69, n. 9, p. 3826-3837, 1991. ISSN 0021-8812.

COUTINHO, E. M. Gossypol: a contraceptive for men. **Contraception**, Los Altos, v. 65, n. 4, p. 259-263, 2002. ISSN 0010-7824.

CRAWFORD, M. The role of dietary fatty-acids in biology - their place in the evolution of the human brain. **Nutrition Reviews**, Mailen, v. 50, n. 4, p. 3-11, 1992. ISSN 0029-6643.

CUSACK, P.; PERRY, V. The effect of feeding whole cottonseed on the fertility of bulls. **Australian Veterinary Journal**, Queensland, v. 72, n. 12, p. 463-466, 1995. ISSN 0005-0423.

DANKE, R. J.; PANCIERA, R. J.; TILLMAN, A. D. Gossypol toxicity studies with sheep. **Journal of Animal Science**, Albany, v. 24, n. 4, p. 1199-1201, 1965. ISSN 0021-8812.

EL-SHARAKY, A. et al. Spermatotoxicity, biochemical changes and histological alteration induced by gossypol in testicular and hepatic tissues of male rats. **Food and Chemical Toxicology**, London, v. 48, n. 12, p. 3354-3361, 2010. ISSN 0278-6915.

FRENCH, P. et al. Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. **Journal of Animal Science**, Albany, v. 78, n. 11, p. 2849-2855, 2000. ISSN 0021-8812.

FTHENAKIS, G. C. et al. Case report: high prevalence rate of ovine mastitis, caused by coagulase-negative staphylococci and predisposed by increased gossypol consumption. **Small Ruminant Research**, Amsterdam, v. 52, n. 1-2, p. 185-189, 2004. ISSN 0921-4488.

GAMBOA, D. et al. Use of expander cottonseed meal in broiler diets formulated on a digestible amino acid basis. **Poultry Science**, Champaign v. 80, n. 6, p. 789-794, 2001. ISSN 0032-5791.

GIOIELLI, L. A. Óleos e gorduras vegetais: composição e tecnologia. **Revista Brasileira de Farmacognosia**, Curitiba, v. 5, n. 2, p. 211-232, 1996.

GIZEJEWSKI, Z. et al. Cottonseed feeding delivers sufficient quantities of gossypol as a male deer contraceptive. **European Journal of Wildlife Research**, Heidelberg, v. 54, n. 3, p. 469-477, 2008. ISSN 1612-4642.

HRON, R.; KUK, M.; ABRAHAM, G. Determination of free and total gossypol by high-performance liquid-chromatography. **Journal of the American Oil Chemists Society**, Chicago, v. 67, n. 3, p. 182-187, 1990. ISSN 0003-021X.

JANAUN, J.; ELLIS, N. Perspectives on biodiesel as a sustainable fuel. **Renewable & Sustainable Energy Reviews**, Amsterdam, v. 14, n. 4, p. 1312-1320, 2010. ISSN 1364-0321.

JARQUIN, R. et al. Effect of cooking and calcium and iron supplementation on gossypol toxicity in swine. **Journal of Agricultural and Food Chemistry**, Easton, v. 14, n. 3, p. 275-279, 1966. ISSN 0021-8561.

JIMENEZ, D. A. et al. Effect of feeding gossypol in cottonseed meal on growth, semen quality, and spermatogenesis of yearling Holstein bulls. **Journal of Dairy Science**, Lancaster, v. 72, n. 7, p. 1866-75, 1989. ISSN 0022-0302.

KANDYLIS, K.; NIKOKYRIS, P. N.; DELIGIANNIS, K. Performance of growing-fattening lambs fed whole cotton seed. **Journal of the Science of Food and Agriculture**, London, v. 78, n. 2, p. 281-289, 1998. ISSN 0022-5142.

_____. Performance of growing-fattening lambs fed diets containing different proportions of cotton seed meal. **Journal of the Science of Food and Agriculture**, London, v. 79, n. 12, p. 1613-1619, 1999. ISSN 0022-5142.

KIM, H.; CALHOUN, M.; STIPANOVIC, R. Accumulation of gossypol enantiomers in ovine tissues. **Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology**, Oxford, v. 113, n. 2, p. 417-420, 1996. ISSN 0305-0491.

KIM, S. et al. Effects of dietary n-6 : n-3 fatty acid ratio on feed intake, digestibility, and fatty acid profiles of the ruminal contents, liver, and muscle of growing lambs. **Journal of Animal Science**, Albany, v. 85, n. 3, p. 706-716, 2007. ISSN 0021-8812.

KOCHMAN, K. Key role of omega-3 and omega-6 fatty acids in human biology; Crawford's predictive theory of evolution. **Postepy Biologii Komorki**, Poznan, v. 39, n. 2, p. 189-198, 2012. ISSN 0324-833X.

LABORDE, F. et al. Breed effects on growth performance, carcass characteristics, fatty acid composition, and palatability attributes in finishing steers. **Journal of Animal Science**, Albany, v. 79, n. 2, p. 355-365, 2001. ISSN 0021-8812.

LAUGERETTE, F. et al. Oil composition of high-fat diet affects metabolic inflammation differently in connection with endotoxin receptors in mice. **American Journal of Physiology-Endocrinology and Metabolism**, Bethesda, v. 302, n. 3, p. E374-E386, 2012. ISSN 0193-1849.

MADRUGA, M. et al. Effect of diets with increasing levels of whole cotton seed on chemical composition and fatty acid profile of Santa Inez (Santa Ines) lamb meat. **Revista Brasileira de Zootecnia**, Viçosa, v. 37, n. 8, p. 1496-1502, 2008. ISSN 1516-3598.

MAGALHAES, K. et al. Effects of feeding different levels of cottonseed hulls on production and carcass physical composition and traits of feedlot steers. **Revista Brasileira de Zootecnia**, Viçosa, v. 34, n. 6, p. 2466-2474, 2005. ISSN 1516-3598.

MAIA, F. et al. Feeding vegetable oil to lactating goats: milk production and composition and milk fatty acids profile. **Revista Brasileira de Zootecnia**, Viçosa, v. 35, n. 4, p. 1504-1513, 2006. ISSN 1516-3598.

MCCAUGHEY, K. et al. Impact of feeding whole Upland cottonseed, with or without cracked Pima cottonseed with increasing addition of iron sulfate, on productivity and plasma gossypol of lactating dairy cattle. **Animal Feed Science and Technology**, Amsterdam, v. 122, n. 3-4, p. 241-256, 2005. ISSN 0377-8401.

MENA, H. et al. The effects of feeding varying amounts of gossypol from whole cottonseed and cottonseed meal in lactating dairy cows. **Journal of Dairy Science**, Lancaster, v. 84, n. 10, p. 2231-2239, 2001. ISSN 0022-0302.

MICINSKI, J. et al. Health-supporting properties of beef. **Journal of Elementology**, Olsztyn, v. 17, n. 1, p. 149-157, 2012. ISSN 1644-2296.

NAGALAKSHMI, D.; SASTRY, V.; PAWDE, A. Rumen fermentation patterns and nutrient digestion in lambs fed cottonseed meal supplemental diets. **Animal Feed Science and Technology**, Amsterdam, v. 103, n. 1-4, p. 1-14, 2003. ISSN 0377-8401.

NIKOKYRIS, P. N.; KANDYLIS, K.; DELIGIANNIS, K. Effects of varying levels of dietary free gossypol in whole cotton seed on physiological responses of growing-fattening lambs. **Journal of the Science of Food and Agriculture**, London, v. 79, n. 14, p. 1969-1981, 1999. ISSN 0022-5142.

NOFTSGER, S. et al. Effect of whole and expanded-expelled cottonseed on milk yield and blood gossypol. **Journal of Dairy Science**, Lancaster, v. 83, n. 11, p. 2539-2547, 2000. ISSN 0022-0302.

NRC. National Research Council. **Nutrient requirements of small ruminants:** sheep, goats, cervids, and new world camelids. Washington, DC: The National Academies Press, 2007. ISBN 9780309102131. Disponível em: http://www.nap.edu/openbook.php?record_id=11654.

OKO, R.; HRUDKA, F. Effect of gossypol on spermatozoa. **Contraceptive Delivery Systems**, Lancaster, v. 3, n. 3-4, p. 281-281, 1982a. ISSN 0143-6112.

_____. Segmental aplasia of the mitochondrial sheath and sequelae induced by gossypol in rat spermatozoa. **Biology of Reproduction**, Champaign, v. 26, n. 1, p. 183-195, 1982b. ISSN 0006-3363.

OLIVEIRA, D. et al. Fatty acid profile and qualitative characteristics of meat from zebu steers fed with different oilseeds. **Journal of Animal Science**, Albany, v. 89, n. 8, p. 2546-2555, 2011. ISSN 0021-8812.

OLIVEIRA, E. et al. Quality traits and lipid composition of meat from Nellore young bulls fed with different oils either protected or unprotected from rumen degradation. **Meat Science**, Barking, v. 90, n. 1, p. 28-35, 2012. ISSN 0309-1740.

PAIM, T. P. et al. Uso de subprodutos do algodão na nutrição de ruminantes. **Ciência Veterinária nos Trópicos**, Recife, v. 13, p. 24-37, 2010.

PEREIRA, M. et al. The renewable energy market in Brazil: Current status and potential. **Renewable & Sustainable Energy Reviews**, Amsterdam, v. 16, n. 6, p. 3786-3802, 2012. ISSN 1364-0321.

PERFIELD II, J.W. et al. Trans-9, Cis-11 Conjugated Linoleic Acid Reduces Milk Fat Synthesis in Lactating Dairy Cows. **Journal of Dairy Science**, Lancaster, v.90, 2211-2218, 2007.

PIPEROVA, L. et al. Mammary lipogenic enzyme activity, trans fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. **Journal of Nutrition**, Bethesda, v. 130, n. 10, p. 2568-2574, 2000. ISSN 0022-3166.

PIRES, A. et al. Effects of heat treatment and physical processing of cottonseed on nutrient digestibility and production performance by lactating cows. **Journal of Dairy Science**, Lancaster, v. 80, n. 8, p. 1685-1694, 1997. ISSN 0022-0302.

RAES, K.; DE SMET, S.; DEMEYER, D. Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. **Animal Feed Science and Technology**, Amsterdam, v. 113, n. 1-4, p. 199-221, 2004. ISSN 0377-8401.

RANDEL, R.; CHASE, C.; WYSE, S. Effects of gossypol and cottonseed products on reproduction of mammals. **Journal of Animal Science**, Albany, v. 70, n. 5, p. 1628-1638, 1992. ISSN 0021-8812.

ROBINSON, P. et al. Influence of variety and storage for up to 22 days on nutrient composition and gossypol level of Pima cottonseed (Gossypium spp.). **Animal Feed Science and Technology**, Amsterdam, v. 91, n. 3-4, p. 149-156, 2001. ISSN 0377-8401.

ROBINSON, R. et al. Effects of dietary polyunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. **Reproduction**, Cambridge, v. 124, n. 1, p. 119-131, 2002. ISSN 1470-1626.

ROGERS, G.; POORE, M.; PASCHAL, J. Feeding cotton products to cattle. **Veterinary Clinics of North America-Food Animal Practice**, Philadelphia, v. 18, n. 2, p. 267-294, 2002. ISSN 0749-0720.

ROGÉRIO, M. C. P. et al. Uso do caroço de algodão na alimentação de ruminantes. **Arquivo de Ciência Veterinária e Zoologia UNIPAR**, Umuarama, v. 56, n. 5, p. 665-670, 2003.

_____. Efeito do nível de caroço de algodão sobre a digestibilidade da fibra dietética do feno de Tifton 85 (Cynodon spp.) em ovinos. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v. 56, p. 665-670, 2004. ISSN 0102-0935.

ROMANO, G.; SCHEFFLER, J. Lowering seed gossypol content in glanded cotton (Gossypium hirsutum L.) lines. **Plant Breeding**, Berlin, v. 127, n. 6, p. 619-624, 2008. ISSN 0179-9541.

SANTOS, I. C. D. S. et al. Beneficiamento de algodão orgânico no agreste paraibano. In: CONGRESSO BRASILEIRO DO ALGODÃO, 7., 2009, Foz do Iguaçu. **Sustentabilidade da cotonicultura brasileira e expansão dos mercados**; anais... Campina Grande: Embrapa Algodão, 2009. p. 50-55.

SANTOS, M. D. et al. Morfologia testicular e qualidade espermática de touros da raça Nelore, submetidos à dieta contendo gossipol. **Revista Brasileira de Ciência Veterinária**, Niterói, v. 15, n. 3, p. 134-139, 2008.

SARTORI, R.; GUARDIEIRO, M. M. Fatores nutricionais associados à reprodução da fêmea bovina. **Revista Brasileira de Zootecnia**, Viçosa, v. 39, p. 422-432, 2010. ISSN 1516-3598.

SIMOPOULOS, A. Evolutionary aspects of diet, essential fatty acids and cardiovascular disease. **European Heart Journal Supplements**, Oxford, v. 3, n. D, p. D8-D21, 2001. ISSN 1520-765X.

_____. The importance of the ratio of omega-6/omega-3 essential fatty acids. **Biomedicine & Pharmacotherapy**, Paris, v. 56, n. 8, p. 365-379, 2002. ISSN 0753-3322.

TALIPOV, S. et al. The Crystal and Molecular Structure of Dianhydrogossypol. **Journal of the American Oil Chemists' Society**, Chicago, v. 86, n. 3, p. 207-213, 2009. ISSN 0003-021X.

TILYABAEV, K. et al. Crystal Structures of the Solvates of Di-ethylaminogossypol with Ethyl Acetate and Pyridine. **Journal of Chemical Crystallography**, New York, v. 39, n. 9, p. 677-682, 2009. ISSN 1074-1542.

VALADARES FILHO, S. D. C. et al. **Exigências nutricionais de zebuínos puros e cruzados**. 2. ed. Viçosa: UFV, 2010. 193 p.

WADHWA, M.; MAKKAR, G.; ICHHPONANI, J. Disappearance of protein supplements and their fractions in sacco. **Animal Feed Science and Technology**, Amsterdam, v. 40, n. 4, p. 285-293, 1993. ISSN 0377-8401.

WANG, X.; PLHAK, L. Production, characterization, and application of anti-gossypol polyclonal antibodies. **Journal of Agricultural and Food Chemistry**, Easton, v. 48, n. 11, p. 5109-5116, 2000. ISSN 0021-8561.

_____. Monoclonal antibodies for the analysis of gossypol in cottonseed products. **Journal of Agricultural and Food Chemistry**, Easton, v. 52, n. 4, p. 709-712, 2004. ISSN 0021-8561.

WILLARD, S. et al. Effects of free gossypol in the diet of pregnant and postpartum brahman cows on calf development cow performance. **Journal of Animal Science**, Albany, v. 73, n. 2, p. 496-507, 1995. ISSN 0021-8812.

YU, F. et al. Effect of heat treatment upon the chemical composition of cottonseed meal and upon the reactivity of cottonseed condensed tannins. **Journal of the Science of Food and Agriculture**, London, v. 72, n. 2, p. 263-272, 1996. ISSN 0022-5142.

ZHANG, L. et al. Chiral gossypol derivatives: Evaluation of their anticancer activity and molecular modeling. **European Journal of Medicinal Chemistry**, Paris, v. 44, n. 10, p. 3961-3972, 2009. ISSN 0223-5234.

ZHANG, W. et al. Advances in gossypol toxicity and processing effects of whole cottonseed in dairy cows feeding. **Livestock Science**, Amsterdam, v. 111, n. 1-2, p. 1-9, 2007. ISSN 1871-1413.

_____. Development of a microbial fermentation process for detoxification of gossypol in cottonseed meal. **Animal Feed Science and Technology**, Amsterdam, v. 135, n. 1-2, p. 176-186, 2007. ISSN 0377-8401.

ZINN, R.; PLASCENCIA, A. Interaction of whole cottonseed and supplemental fat on digestive function in cattle. **Journal of Animal Science**, Albany, v. 71, n. 1, p. 11-17, 1993. ISSN 0021-8812.

4. IMPACT OF FEEDING COTTONSEED CO-PRODUCTS ON REPRODUCTIVE SYSTEM OF PUBERTAL LAMBS

Abstract

The aim of this study was to investigate the effect of feeding three cotton co-products with different gossypol concentrations on reproductive development of lambs close to puberty. Twenty four 5-months old ram lambs were used. These received four diets: 20% of dry matter intake (DMI) of whole cottonseed (WCS), 20%DMI of cottonseed meal (CSM), 20%DMI of high oil cottonseed meal (CSC) and a control group (CTL) without cottonseed co-products. Free gossypol intake was 0, 16.32, 6.98 and 5.47 mg/kgBW for CTL, WCS, CSC and CSM, respectively. At each 15 days, the animals were weighted and semen samples were collected. It was analyzed sperm motility, vigor, mass movement, concentration and pathologies. The free testosterone and cortisol concentrations in serum were determined. After 95 experimental days, the animals were slaughtered and testis samples were collected to analysis in light and transmission electron microscopes. The treatments did not differ in average daily gain, ejaculated volume, sperm motility, vigor and concentration. The CTL group had higher testosterone concentration than CSC at the end of trial. CTL group also had lower total sperm defects, higher mass movement and better seminiferous epithelium structure than others groups. The number of mitochondrial sheath aplasia increased with increasing gossypol level in diet. Therefore, the gossypol induces this lesion. There was no relation between the other variables evaluated and gossypol level in diets; however the groups that received cottonseed co-products showed worse reproductive parameters than CTL. Cottonseed co-products have negative impact on reproductive system of puberal lambs regardless gossypol concentration.

Keywords: Gossypol. Sperm. Sheep. Testis. Testosterone.

4.1. Introduction

The Brazilian cottonseed production in 2011 was equal to 1.992.600 t and it is expected that the production will grow at 3.3% per year until 2022 (BRASIL, 2012). Therefore, whole cottonseed and its co-products stand out as an alternative source of protein and energy, which can decrease the cost of the animal diet.

However, a major limitation for using these co-products in animal nutrition is the presence of high levels of gossypol. This is a highly reactive substance, capable of acting as a phenolic and as an aldehydic compound. The phenolic groups of gossypol react readily to form esters and ethers; the aldehyde groups react with amines to form Schiff's bases, and with organic acids to form heat labile compounds (EL-SHARAKY et al., 2010). Studies with humans, monkeys and rats indicate that gossypol can have particular effects upon the male reproductive system, resulting in impaired spermatogenesis and reduced sperm motility (RANDEL; CHASE; WYSE, 1992). The latter effect has been associated with a specific lesion in sperm midpiece, namely the segmental aplasia of the mitochondrial sheath, which has been identified as a consistent feature of gossypol administration (OKO and HRUDKA, 1982; CHENOWETH et al., 2000). The testis appears to be more sensitive and vulnerable to gossypol than other organs, although some authors state that gossypol can directly affect epididymal spermatozoa (DE ANDRADE et al., 2006). The gossypol effect on endocrine system is not yet clear. And gossypol is considered a promissory drug to human male contraception (COUTINHO, 2002).

The gossypol toxicity is considered low in ruminants, because the rumen environment promotes binding of free gossypol to proteins, which turns it physiologically inactive (REISER; FU, 1962). However, studies with ruminants show a wide variety of results, since minimal (CHASE et al., 1994) to severe pathologies (RANDEL; CHASE; WYSE, 1992; CHENOWETH et al., 1994). Moreover, whole cottonseed fed to deer was considered an efficient male contraceptive due to full recovery of semen quality in the year following the treatment (GIZEJEWSKI et al., 2008).

Some studies using cotton co-products failed to demonstrate a relation between higher gossypol concentration and more severe seminal pathologies. This may indicate that the availability of free gossypol may differ between feedstuffs (CHASE et al., 1994), which highlight the importance of evaluating the impact of different cotton co-products on the reproductive system.

The evaluations of gossypol toxicity and cottonseed co-products feeding have been carried out on postpubertal males. Few studies, mainly using bulls and rats (CHASE et al., 1994; DE ANDRADE et al., 2006) and none with sheep, evaluated the effect of feeding gossypol to prepubertal males. Therefore, we evaluated the effect of feeding three cotton co-products with different gossypol concentrations on reproductive development of lambs close to puberty.

4.2. Material and Methods

Twenty-four Santa Inês lambs males with mean body weight equal to 20.6 ± 1.9 kg and initial age of 5 months were housed in individual covered pens with a concrete floor. These animals were divided equally in four diets: control (without cottonseed co-products, CTL); whole cottonseed (WCS); pressure-extracted high oil cottonseed meal (CSC); solvent-extracted cottonseed meal (CSM) (Table 4.1).

This experiment was carried out after approval by the university animal ethics committee. The experimental period lasted 95 days and was preceded by an adaptation period of 14 days. The diets were elaborated according to NRC (2007), aiming for a daily body weight gain of 200 g/day (Table 4.1). The proportion concentrate:forage was 50:50 and Coast cross (*Cynodon dactylon* (L.) Pers) hay was used as forage. Mineral salt and urea (27 g/kg) were added to concentrate in the same amount for all groups. Soybean oil was added to the concentrate in CTL, CSM and CSC to equalize ether extract. The animals were fed twice daily, morning (8 h) and afternoon (17 h). The amount of feed offered was adjusted according to animal consumption, to achieve ort equal to 10% of offer. The determination of free gossypol concentrations in diet were realized through spectrophotometry UV-VIS method according to Wang (1987), adapted and optimized.

Animals were weighed fortnightly during the experiment, after 10 hours fasting. On the same days at 9 h, the blood samples were collected to determine cortisol and free testosterone levels employing radioimmunoassay commercial kits (DPC Medlab Coat-A-Count). Cortisol was used as an indicator of metabolic rate, as a low metabolic rate could affect testosterone levels and other experimental results. The raw data in cpm (counts per minute) obtained from gamma counter were analyzed by the logit-log plot develop by

Rodbard and Lewald[1] (1970 apud GEIGER, 1992). All samples and standards were measured in duplicates. The ranges of calibration curves were 0.63 to 52 pg/mL for free testosterone and 1 to 46 µg/100 mL for cortisol.

Table 4.1 - Composition of concentrates, free gossypol content in concentrates and concentrate formulation

	Hay	**CTL**	**WCS**	**CSM**	**CSC**
Dry matter (%)	90.78	90.96	91.88	91.84	91.5
Crude protein (%)	5.30	24.08	25.29	23.53	22.63
Neutral detergent fiber (%)	86.09	70.68	70.64	69.17	65.98
Acid detergent fiber(%)	52.90	17.73	28.41	27.01	26.27
Ether extract (%)	2.17	8.15	10.69	11.04	13.66
Ash content (%)	8.55	5.6	5.86	5.43	6.16
Free gossypol (mg/kg)	0	0	1020	350	430
Concentrate formulation					
Ingredients		**CTL**	**WCS**	**CSM**	**CSC**
Ground corn (g/kg)		642	341	424	392
Soybean meal (g/kg)		331	242	159	191
Whole cottonseed (g/kg)		0	390	0	0
Cottonseed meal (g/kg)		0	0	390	0
Cottonseed cake (g/kg)		0	0	0	390
Soybean oil (ml/kg)		87	0	92.5	74.5
Urea (g/kg)		27	27	27	27

CTL: without cottonseed co-products in diet; WCS: 20% of whole cottonseed in diet; CSM: 20% of cottonseed meal in diet; CSC: 20% of high oil cottonseed meal in diet. Bromatological analysis were carried out for dry matter (DM), crude protein (CP), ether extract (EE) and ash content (A) using Association of Official Agricultural Chemistry (1995) procedures and neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Mertens et al. (2002).

[1] RODBARD, D.; LEWALD, J. Computer analysis of radioligand assay and radioimmunoassay data. **Acta Endocrinologica**, p. 79-&, 1970. ISSN 0001-5598.

Also fortnightly, measures of scrotal size (circumference, length and width) were carried out. Semen samples were collected through eletroejaculation and seminal parameters evaluated according to Manual for Andrological Examination and Semen Evaluation (BCAR − Brazilian College of Animal Reproduction). The volume and aspect of semen were determined by observation in the collector tube. Then, in light microscopy, fresh sperm drop was evaluated for progressive motility (%), vigor (0 to 5) and mass movement (0 to 5). Semen samples were storage in buffered formal-saline (1%) to evaluate sperm morphology and concentration in a contrast phase microscope (wet solution, 1000x). Sperm concentration was performed using a Neubauer chamber, adjusting the final result to the dilution used. Morphology was evaluated in sperm smears looking at 200 spermatozoa per sample to determine the proportion of abnormal spermatozoa: head shape defects (underdeveloped, irregular contour, neck, small and large heads), acrosome defects (reacted, vesicular, detached and bent), midpiece (cracked, gap, bent, thick, notch, broken, abaxial, irregular and pseudodroplet), and tail defects (simple and strongly bent, coiled, proximal and distal droplet as well as coiled around head) (LARSEN; CHENOWETH, 1990; CHENOWETH et al., 1994).

At the end of experimental period, the animals were slaughtered and testis samples were collected. Four samples from each testis were stored in formal solution (10%), then embedded in paraffin, cut at 3 µm thickness and stained with hematoxylin and eosin. Digital capture of images was performed in AxioSkop Microscope (Zeiss) coupled with a colored digital CCD camera (Sony, I model DXC-107A). A total of five photos were taken per animal and, in each photo, five randomly chosen seminiferous tubules were analyzed, totalizing 25 tubules analyzed per animal. The diameter of tubule and lumen were measured in two points of each seminiferous tubule, using the software MICAM® (version 1.4). The seminiferous epithelium thickness was obtained by the difference between the lumen and tubule diameters (Figure 4.1).

Two testis samples (1 mm thick) of each animal were fixed in Karnovsky's fixative (25% glutaraldehyde, 8% paraformaldehyde, 0.2 M phosphate buffer, pH 7.2) and stored at 4 °C. Samples were rinsed in cacodylate buffer, cut into smaller pieces and pos-treated in 2% osmium tetroxide, for 1 hour, and uranyl acetate overnight at 4 °C. Tissue blocks were then dehydrated by exposure to graded concentrations of acetone (30, 50, 70, 90 and 100%) and embedded in Spurr® resin (low viscosity). The ultrathin sections (80 nm) were placed on uncoated copper grids (200 mesh), counterstained with uranyl acetate and lead citrate. The sections were examined in a Zeiss EM 900 transmission electron microscope at 80 kV. Image

analysis was carried out given three scores for each grid, without previous knowledge of animal identification to avoid bias. Sertoli cells were scored (1 to 10) according to shape, size, vacuolization degree, mitochondria and others abnormalities. Spermatogonial cell lineage (spermatogonia, spermatocytes and spermatids) received a score (1 to 10) considering the presence of layers representing the seminiferous cycle stages (WROBEL; REICHOLD; SCHIMMEL, 1995; STEGER; WROBEL, 1996) and the integrity of cells. Lower scores mean a high number of defects. The spermatids in tubule lumen were evaluated for presence or absence (1 or 0) of segmental aplasia of the mitochondrial sheath (SAMT) which is considered a gossypol-induced sperm abnormality (OKO; HRUDKA, 1982; CHENOWETH et al., 2000).

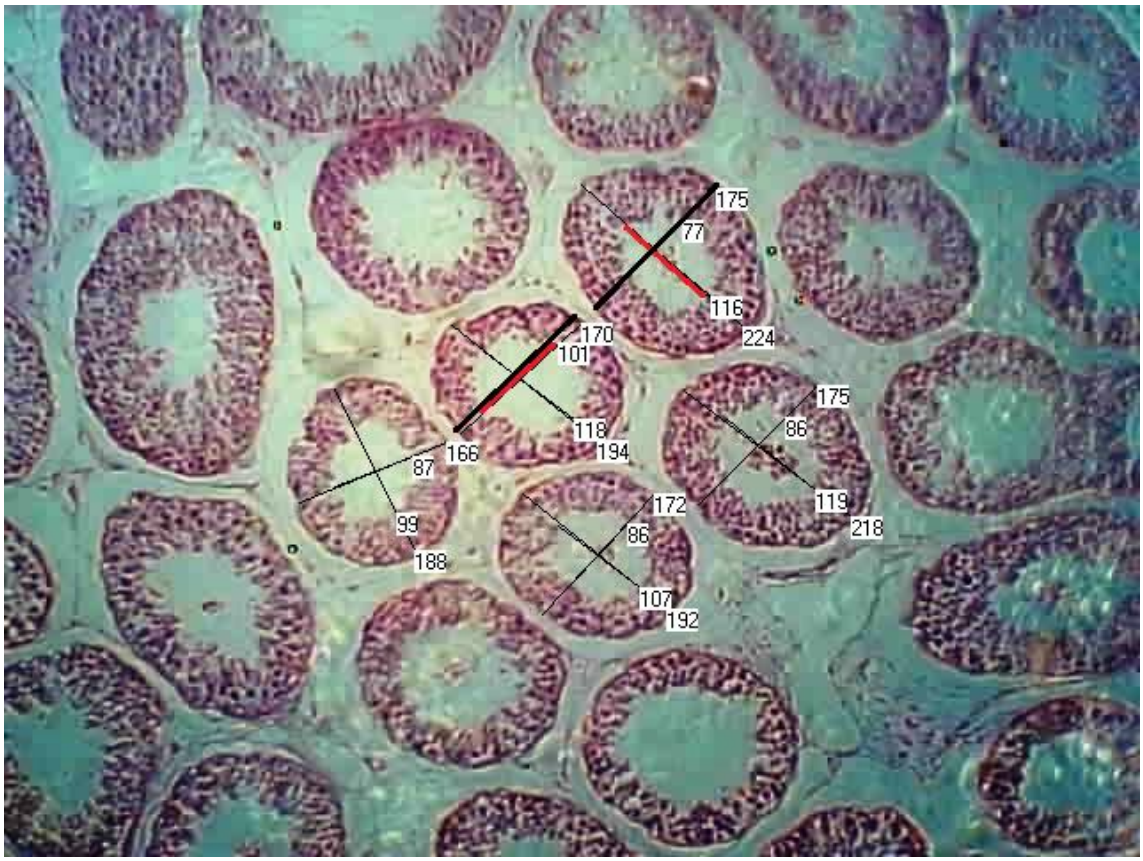

Figure 4.1 - Seminiferous tubule measures in photos taken through light microscopy from testis of lambs. Black line: tubule diameter measure; Red line: lumen diameter measure

Semen from the last collection in the experimental period also was collected for evaluation in transmission electron microscopy (TEM). The preparation procedure was similar to that used for testis samples, exception that a prior step was carried out where semen was embedded in a polymer. Spermatozoa were given a score (1 to 10) according to number of abnormalities found (SIS). The highest score (10) means that no sperm defects were found. The mitochondrial sheaths were analyzed to identify the segmental aplasia (SAMS), so if this lesion was identified the grid received "1" and, if not, "0".

The average daily weight gain (DWG) was obtained by linear regression of weight per day of experiment by each animal and angular coefficient (b) was used in the analysis of variance using the GLM procedure of SAS®. DWG was submitted to a regression analysis considering gossypol level, testing linear and quadratic effects. The hormone results were analyzed as repeated measures using MIXED procedure. Treatment (t), days on experiment (d) and interaction between them (t*d) were used as fixed effects and animal as subject.

Vigor and mass movement were analyzed by Kruskal-Wallis test using the NPAR1WAY procedure of SAS®. The sperm pathologies were analyzed by GLM procedure fitting as fixed effects: t, d and t*d; and as random effect: animal (inside treatment). Sperm volume, motility, concentration and scrotal size (circumference, length and width) were analyzed as repeated measures using the MIXED procedure. A regression analysis between these traits and gossypol level in diet (linear and quadratic effect) was carried out. Finally, a factor analysis was used to verify the relation between these variables.

Seminiferous tubules measures were submitted to variance analyses, using treatment, photo, tubule inside photo, final weight of animal as fixed effects. Correlations, regression with gossypol level in diet, and factor analysis were carried out. The scores obtained in testis and semen samples observed in TEM were evaluated using a logistic regression testing treatment effect and also effect of gossypol level in diet. Correlation and factor analysis also were carried out to this data.

Statistical analyses were carried out using SAS® (Statistical Analysis System, 9.0). Normality of residuals and homogeneity of variance tests were performed on raw data. Transformations were carried out when necessary. The results of sperm pathologies and motility (percentages) were transformed using the arc sine of square root. In all mixed model analyzes, the covariance structure that better adjusted to data was chosen according to the lowest AICC and BIC. In all analyzes of variance and repeated measures analyses, means were compared through least square means when $p<0.05$, using pdiff statement in SAS®.

4.3. Results

The estimated gossypol intake per treatment was 0, 16.32, 6.98 and 5.47 mg/kg of Body Weight (BW) for CTL, WCS, CSC and CSM, respectively. The average daily weight gain (DWG) was 138.63 g/day (±7.95), not differing between treatments. Regression analysis showed no significant effect of gossypol level in diet.

The volume ejaculated, sperm concentration and motility increased as animals grew, but was not affected by treatments (Figure 4.2). The scrotal size measures (circumference, length and wide) also increased according with time without treatment effects. The regressions of these traits with gossypol level in diet were not significant. The factors analysis also showed that gossypol level was not related to these traits.

Sperm vigor was not affected by treatments. On the other hand, the animals from the CTL group showed higher score for mass movement than the others groups. The animals from CSM and CSC groups had more head defects and WCS and CSM groups had higher number of coiled tail compared to CTL group. And the CTL group had the lowest total sperm defects (Table 4.2). The factors analysis showed that gossypol level in the diet was not related to any sperm pathologies (Figure 4.3).

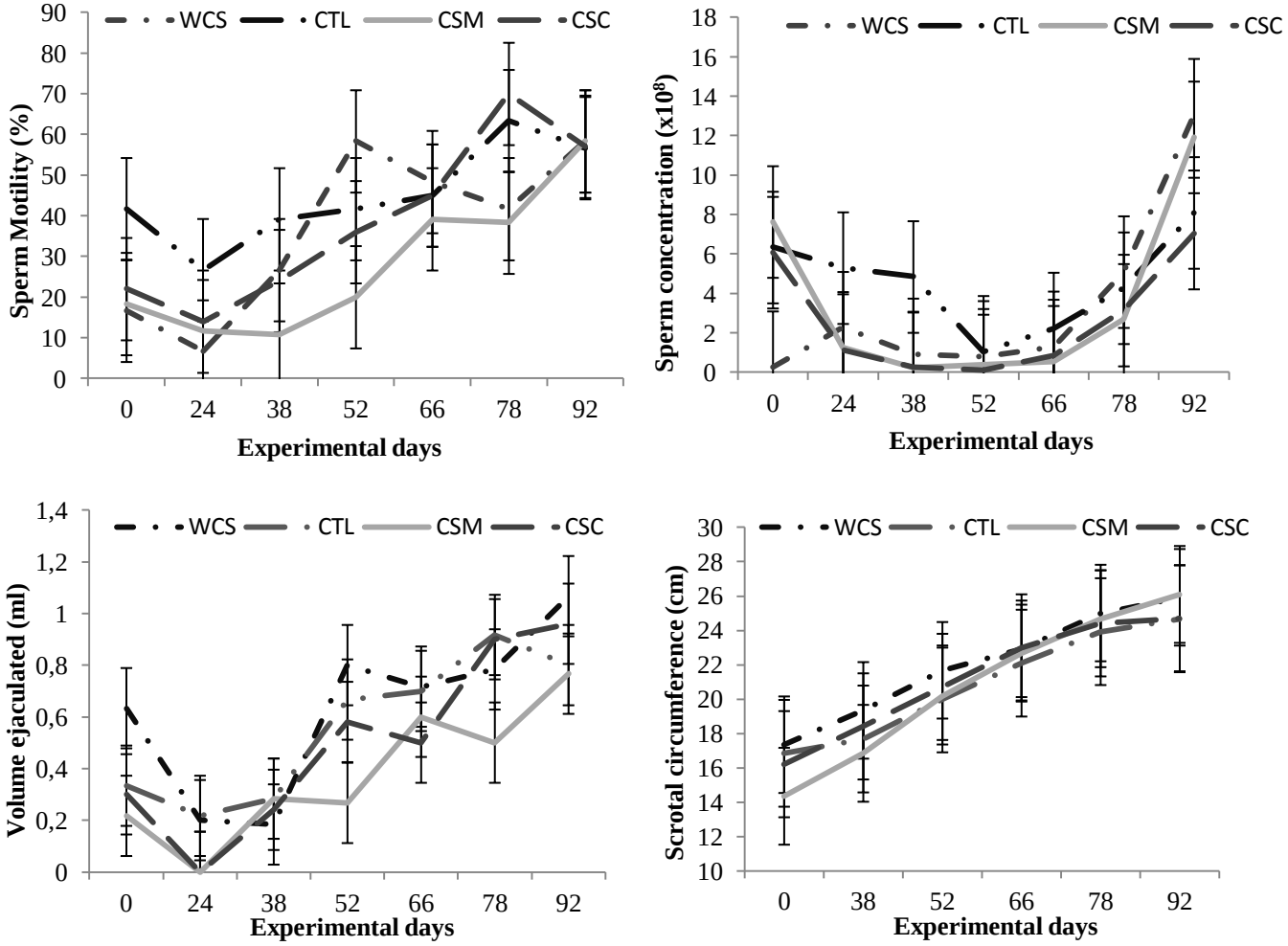

Figure 4.2 - Least square means for volume ejaculated, sperm motility, sperm concentration and scrotal circumference from lambs receiving: whole cottonseed (WCS), high oil cottonseed meal (CSC), low oil cottonseed meal (CSM) and control (CTL) without cottonseed co-products

Table 4.2 - Least square means for sperm defects* (%) from Santa Inês lambs that received whole cottonseed (WCS), high oil cottonseed meal (CSC), low oil cottonseed meal (CSM) and control diet without cotton co-products (CTL)

	CTL	WCS	CSC	CSM	Mean	SD
Head defects						
Acrosome	0.90^{c}	1.99^{bc}	4.03^{a}	2.57^{ab}	1.94	2.28
Isolated	2.72^{b}	4.76^{b}	2.30^{b}	7.18^{a}	3.35	4.89
Thin	1.76^{a}	1.36^{a}	2.60^{a}	2.27^{a}	1.75	1.65
Neck shape	1.63^{b}	0.79^{b}	3.74^{a}	2.77^{a}	1.69	1.64
Midpiece defects						
Folded	0.10^{b}	0.43^{a}	0.16^{ab}	0.21^{ab}	0.11	0.40
Folded at end	1.73^{a}	1.39^{a}	1.99^{a}	1.94^{a}	1.95	3.21
Coiled at end	1.72^{a}	0.33^{a}	2.32^{a}	0.79^{a}	1.41	4.40
Notch	0.024^{b}	0.119^{ab}	0.029^{ab}	0.121^{a}	0.04	0.17
Corkscrew	0.12^{a}	0.05^{ab}	0.13^{a}	0.00^{b}	0.05	0.15
Irregular	0.27^{b}	0.28^{b}	1.04^{a}	0.09^{b}	0.27	0.61
Tail defects						
Simple folded	2.73^{a}	1.11^{a}	1.51^{a}	1.22^{a}	2.49	2.47
Strongly folded	6.85^{a}	10.21^{a}	8.11^{a}	6.66^{a}	8.37	6.74
Coiled	6.82^{b}	15.05^{a}	8.65^{ab}	11.96^{a}	8.77	8.58
Pseudodroplet	0.24^{ab}	0.11^{b}	0.36^{a}	0.06^{b}	0.15	0.42
Proximal cyt. Droplet	5.09^{c}	11.66^{ab}	15.35^{a}	6.02^{bc}	7.04	8.75
Distal cyt. Droplet	2.65^{a}	1.43^{a}	0.93^{a}	1.50^{a}	1.91	3.85
Coiled around head	2.20^{b}	2.59^{ab}	2.04^{b}	4.06^{a}	2.18	2.84
Totals						
Head defects	8.39^{c}	12.02^{bc}	15.04^{ab}	17.32^{a}	10.62	6.83
MP defects	4.18^{a}	3.18^{a}	5.81^{a}	3.43^{a}	4.02	6.85
Tail defects	32.61^{b}	46.55^{a}	43.67^{a}	36.57^{ab}	35.94	16.22
Sperms defects	41.00^{b}	58.58^{a}	58.70^{a}	53.89^{a}	46.56	16.86

*Showed only sperm defects (pathologies) that had differences between treatments or had mean above 1%. Different letters in the same row means statistical difference ($P < 0.05$). SD: standard deviation; cyt.: cytoplasmic. MP defects: total midpiece defects.

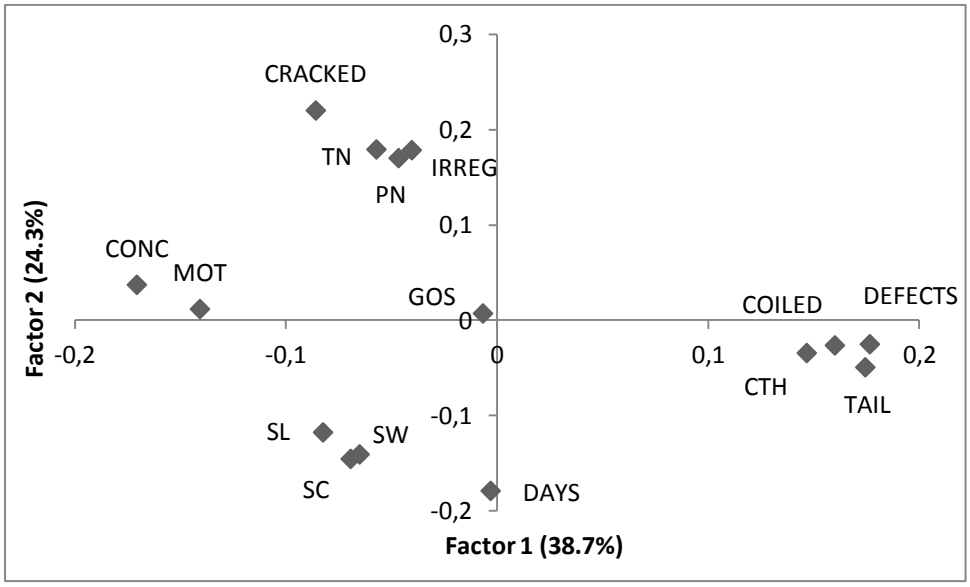

Figure 4.3 - Factor analysis of scrotal size measures, sperm pathologies, gossypol level in diet and immediate sperm traits from Santa Inês lambs fed with whole cottonseed, cottonseed meal with low oil, cottonseed meal with high oil and a control without use of cottonseed during 95 experimental days. The values between parentheses show the amount of variance explained by each factor. CONC: sperm concentration; MOT: sperm motility; SL: scrotal length; SW: scrotal width; SC: scrotal circumference; DAYS: days on experiment; GOS: gossypol level in diets; CRACKED: cracked midpiece; IRREG: irregular midpiece; PN; TN: partially and totally notch midpiece, respectively; COILED: coiled tail; CTH: coiled tail around head; TAIL: total tail defects; DEFECTS: total number of spermatozoa with defects

The seminiferous tubule measures showed a significant effect of treatment, the CSM group having thicker seminiferous epithelium (Table 4.3). However, the regression and factor analyses showed no effect of gossypol level in the diet. The regression analysis demonstrated that thicker seminiferous epithelium determine higher sperm concentrations in the ejaculated semen. There was a significant and positive correlation between the sperm concentration and seminiferous tubule measures ($0.27 < r < 0.44$).

Table 4.3 - Least square means for tubule diameter, lumen diameter and seminiferous epithelium thickness (mm) of lambs fed with whole cottonseed (WCS), cottonseed meal with low oil (CSM), cottonseed meal with high oil (CSC) and a control without use of cottonseed (CTL)

	CTL	WCS	CSC	CSM	Mean	CV(%)
Tubule	200.7[b]	199.3[b]	191.7[c]	204.5[a]	198.7	15.9
Lumen	105.9[a]	104.6[ab]	98.4[c]	103.0[b]	103.7	21.2
Epithelium	94.8[b]	94.7[b]	93.3[b]	101.5[a]	95.0	22.5

Different letters in the same row means significant statistical differences (P < 0.05). CV: coefficient of variation.

Cortisol levels decreased with days on experiment and there was no treatment effect. The free testosterone levels increased with time, which was expected as the animals were in the pubertal period. At 90 days on experiment, the CTL group had higher testosterone level than the CSC group (Figure 4.4).

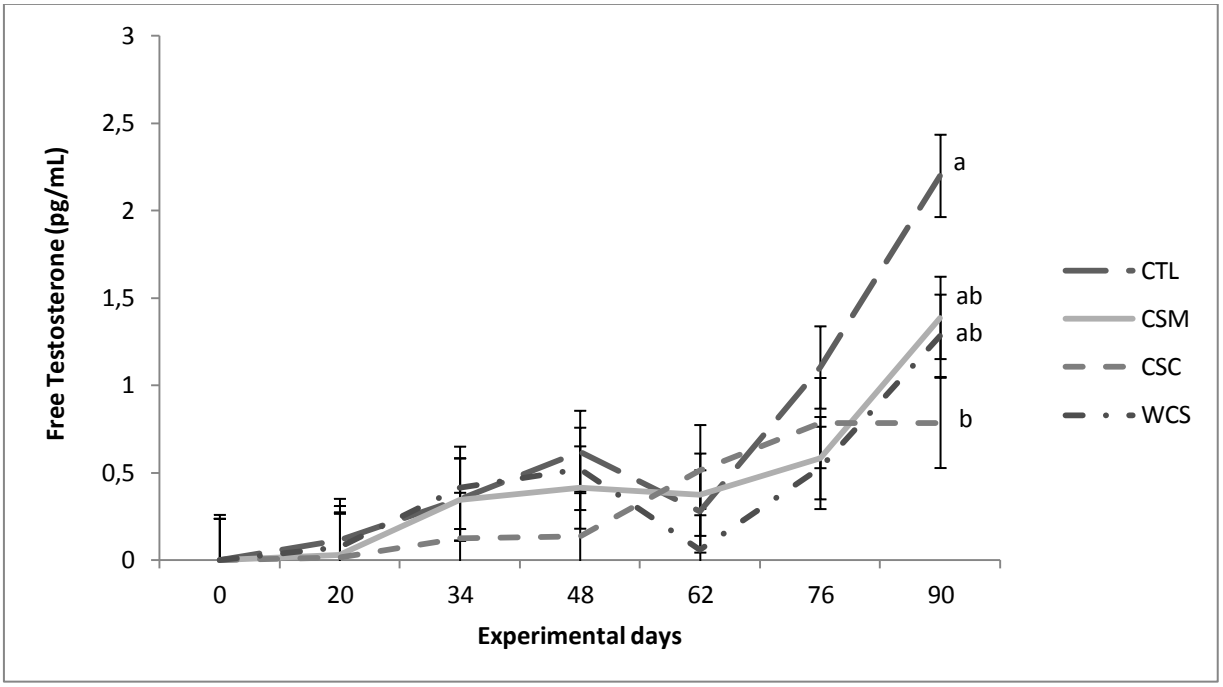

Figure 4.4 - Free testosterone levels in serum from Santa Inês lambs fed with whole cottonseed (WCS), cottonseed meal with low oil (CSM), cottonseed meal with high oil (CSC) and a control without use of cottonseed (CTL). Different letters between lines means statistical significant difference between treatments (P < 0.05)

In relation to spermatozoa observed in TEM, the WCS and CSC group had lower scores for SIS than CTL and CSM groups (odds ratios equal to 3.9 between WCS and CSC vs CTL, and odds ratios equal to 18.6 between WCS and CSC vs CSM). The logistic regression analyzing the SAMS (Figure 4.5) showed that CSM group differentiated from WCS group (odds ratio = 14, modeling for non lesion probability, and p=0.04 in contrast test). The contrast between CTL and CSM vs CSC and WCS also was significant. The contrast test analyzing effects of gossypol level in the diet showed a linear trend (p=0.08). Sixty-seven percent of sperm evaluated from the WCS group had SAMS which was 13% in the CSM group.

There are no differences between treatments and gossypol level in diets for Sertoli cells score. On the other hand, the scores for seminiferous epithelium was higher in the CTL group compared to others (odds ratios = 2.96 to WCS vs CTL, 2.49 to CSC vs CTL and 2.20 to CSM vs CTL). The main alterations observed in seminiferous epithelium were vacuolization inter and intra cellular and lower cell size with decrease in cytoplasmic ground substance, which is similar to found by Hoffer (1983). SAMT were not differentiated between treatments.

The scores for SIS and SAMS showed significant correlations with the seminiferous epithelium thickness (0.62; -0.49, respectively). The factor analysis (Figure 4.6) showed a group of variables formed by seminiferous tubule measures, sperm concentration, Sertoli and SIS scores, opposing SAMS. This demonstrated that thicker epithelium led to higher scores for SIS and lower levels of mitochondrial sheath aplasia.

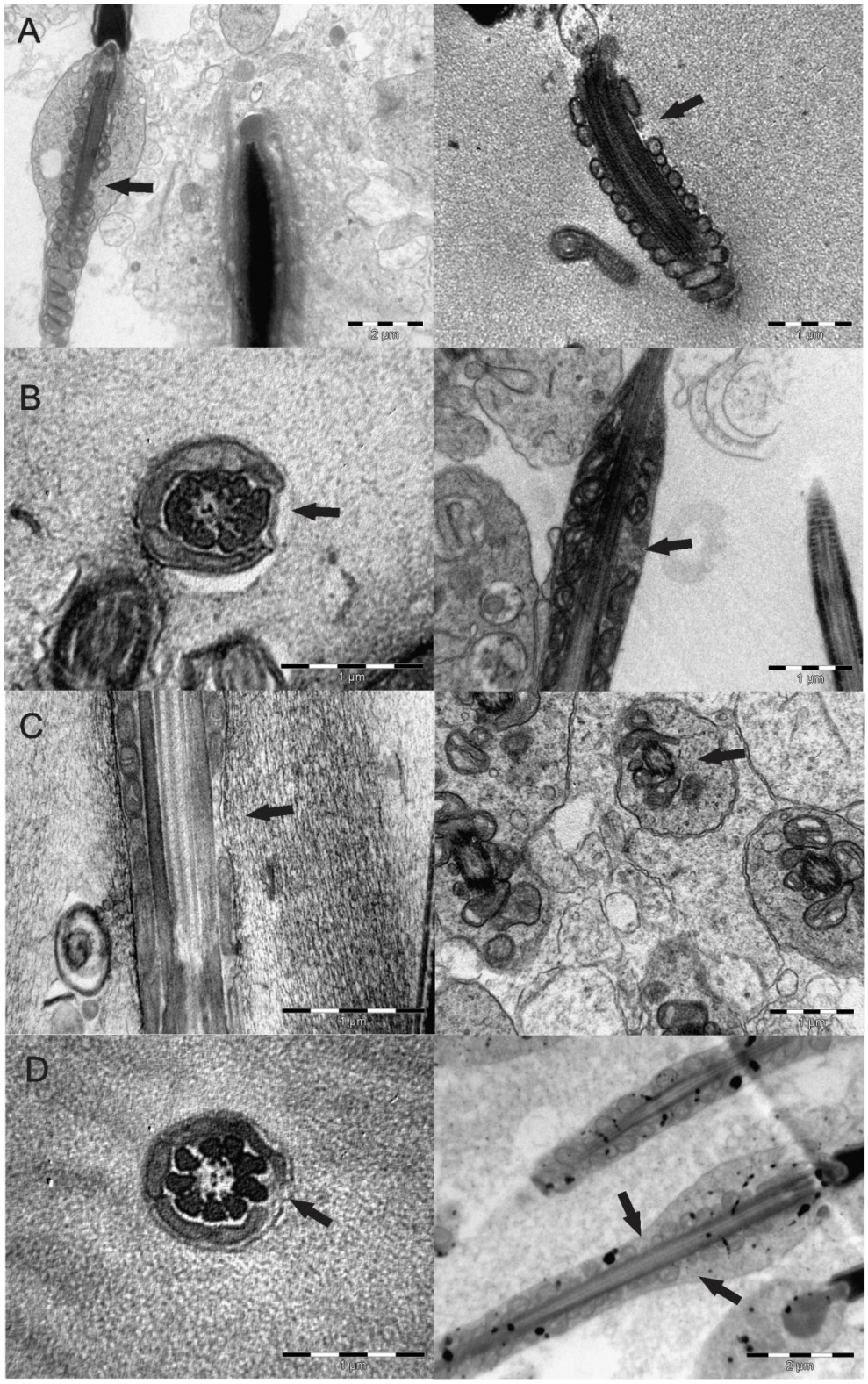

Figure 4.5 - Electron micrographs showing aplasia of mitochondrial sheath (arrows) in spermertazoa from lambs fed with whole cottonseed (A), cottonseed meal with high oil (B), cottonseed meal with low oil (C), and a control without use of cottonseed (D) during 95 experimental days.

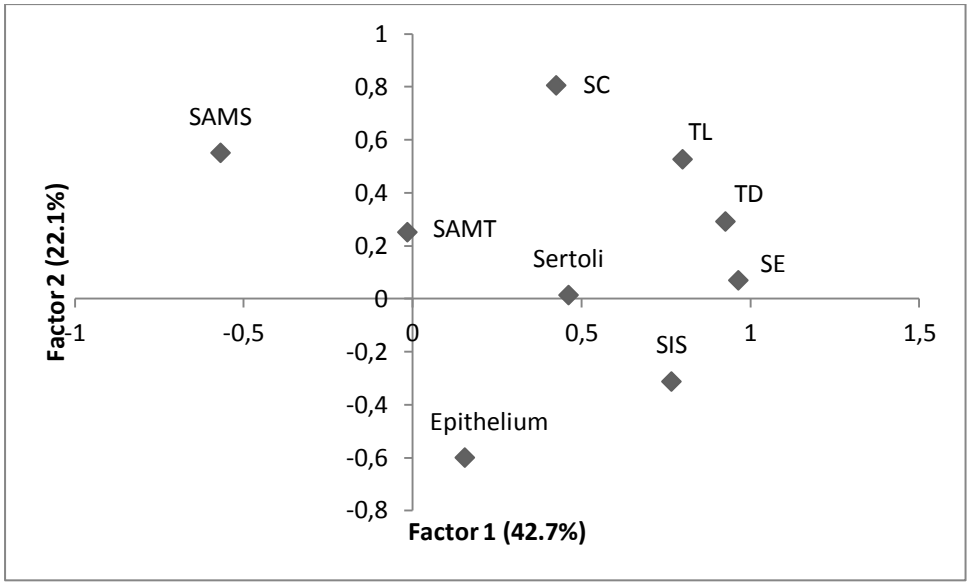

Figure 4.6 - Factor analysis of sperm and testis traits observed in light and transmission electron microscopy from lambs fed with whole cottonseed, cottonseed meal with low oil, cottonseed meal with high oil and a control without use of cottonseed during 95 experimental days. The values between parentheses show the amount of variance explained by each factor. SIS: spermatozoa integrity in semen; SAMS: Segmental aplasia of mitochondrial sheath in spermatozoid observed in semen; Sertoli: score for Sertoli cells in testis; Epithelium: scores for seminiferous epithelium observed in transmission electron microscopy; SAMT: Segmental aplasia of mitochondrial sheath in spermatozoid observed in testis; TD: seminiferous tubule diameter; TL: seminiferous tubule lumen; SE: seminiferous epithelium thickness; SC: sperm concentration

4.4. Discussion

The results showed that feeding up to 16.32 mg/kgBW/day of free gossypol did not impair lamb growth. In a similar study (CHASE et al., 1994), bulls fed whole cottonseed (60 mg/kgBW/day of free gossypol) from weaning through puberty had lower body weight gain and reached puberty at an older age than bulls fed cottonseed meal (6 mg/kgBW/day) or soybean meal. These authors argue that, as puberty was reached at similar body weight and scrotal circumferences among treatments, delayed puberty in bulls fed whole cottonseed may have been due to a lower energy balance and not gossypol per se. Therefore, the main reason for the difference between the studies' results may be related to diet constitution. Our diets had an ether extract (EE) maximum equal to 7.9%, while these other authors utilized next to 10% of EE in whole cottonseed diet, which may impair the fiber digestibility and decrease the forage consumption.

Scrotal size, sperm volume, motility, vigor and concentration were not affected by diets and increased with time showing that animals were in the pubertal phase as expected. In similar studies (CHASE et al., 1994; CHENOWETH et al., 1994), gossypol also did not affect scrotal size, sperm quantity or quality. Other authors found that progressive motility and vigor of sperm cells were influenced by the whole cottonseed, with a downward linear trend as increased the gossypol level in diets (6.8, 9.2 and 11.5 mg/kgBW/day of free gossypol) (CUNHA et al., 2012). Dabrowski et al. (2000) state that the antifertility effect of gossypol is related to how efficiently gossypol crosses a general circulation-gonadal barrier. Therefore, different permeability of reproductive organs due to species, age and also metabolic rate may explain different results of experiments using the similar gossypol dose.

The CSM group had greater tubule diameter, low lumen and, consequently, thicker seminiferous epithelium than others. The gossypol concentration in diets not influenced significantly the seminiferous tubule measures. In a similar study (CHASE et al., 1994), bulls fed cottonseed meal (6 mg/kgBW/day of free gossypol) had greater lumenal diameters, thinner epithelial walls, and fewer germ cell layers than bulls fed whole cottonseed (60 mg/kgBW/day of free gossypol), which would not be expected due to higher free gossypol concentration in the whole cottonseed than meal. In gossypol treated rats, sperm morphology was severely compromised, but the epithelium in testis appeared morphologically normal (HOFFER et al., 1987; DE ANDRADE et al., 2006). Some authors argue that gossypol targets the epididymis, disturbing both the structure and function of this organ, and presumably disrupts sperm maturation without alterations in testis (DE ANDRADE et al., 2006). Therefore, there are controversial results for the impact of gossypol on seminiferous tubule measures. Moreover, the impact of these measures on sperm production and quality are not clear. In the present study, sperm concentration was higher with thicker seminiferous epithelium.

The animals from CTL group had a higher free testosterone concentration at 90 days than CSC group. The gossypol level in diets was not direct related to free testosterone concentration. In similar study, Chase et al. (1994) did not observe differences in testosterone concentrations between puberal bulls fed diets containing gossypol and those fed soybean meal.

The effect of gossypol on the endocrine system is controversial. Several studies showed that gossypol reduced fertility without changes in testosterone, or other androgens, or luteinizing hormone (SHANDILYA et al., 1982; WANG et al., 1984; SOUFIR et al., 1989)

and, consequently, does not decrease sexual potency or libido (COUTINHO, 2002). However, some studies indicated that gossypol has an inhibitory effect on testosterone production by the Leydig cell via a subsequent lesion in pregnenolone formation (OKO; HRUDKA, 1984a; GIZEJEWSKI et al., 2008). In these studies, the antifertility effect of gossypol appears secondary to the decrease of testosterone synthesis (OKO; HRUDKA, 1984a; DE PEYSTER; SREBNIK, 1988; DABROWSKI et al., 2000; EL-SHARAKY et al., 2010) because adult mammalian spermatogenesis is a testosterone dependent process, and many studies have shown that testosterone withdrawal from the rat testis results in increased germ cell apoptosis (BILLIG et al., 1995).

It has been reported that FSH and/or testosterone withdrawal can cause the depletion of mature spermatids through phagocytosis by the Sertoli cells (SAITO et al., 2000). Udoh, Patil and Deshpande (1992) reported that Sertoli and Leydig cells showed progressive regression due to gossypol administration. El-Sharaky et al. (2010) showed significant increases in the activities of testicular 17b-HSD and 17-ketostroid reductase in gossypol treated groups compared to the control group, which may lead to increased degradation of testosterone what may explain the reduction of serum testosterone levels. Moreover, it has been shown that gossypol interferes with key steroidogenic enzymes such as 5a-reductase and 3a-hydroxysteroid dehydrogenase in the rat testis (MOH et al., 1993).

Randel et al. (1992), in a comprehensive review, affirmed that effects of gossypol on testosterone concentrations are not consistent (i.e., no effect or decreased). According to De Andrade et al. (2006), the great contradiction on the endocrinal effects of gossypol may be due to different animal species, different doses and times of treatment, or due to different administration routes.

Sperm pathologies showed that animals from the CTL group had lower total spermatozoa defects, which may be related to this group had better mass movement. The main sperm defects found in animals that receive cotton co-products compared to control group were isolated (detached) heads, coiled tail and proximal cytoplasmic droplet.

In other study (CUNHA et al., 2012) using crescent levels of whole cottonseed (6.8, 9.2 and 11.5 mg/kgBW/day of free gossypol) fed to sheep, the percentage of total defects increased linearly, for each 1% increase of whole cottonseed in the diet, there was an increase of 0.2% in total defects. In the morphological analyses a greater occurrence of defects such as broken acrosome, folded tail, and strongly folded tail were seen (CUNHA et al., 2012).

Chenoweth et al. (1994), studying bulls fed with whole cottonseed (16.4 mg/kgBW/day of free gossypol), observed an increased proportion of sperm midpiece

abnormalities, which stabilized at 52 to 62.5%. In rats that received gossypol daily at a dosage of 15 mg/kgBW/day from weaning through puberty, there was a significant increase in sperm with abnormal morphology in the vas deferens of treated animals, with the most frequent abnormality being the presence of isolated sperm heads (DE ANDRADE et al., 2006).

Our results and those from others studies agree with Oko and Hrudka (1982). According to these authors, there is a common pathogenic path which links many of the major sperm midpiece abnormalities with known spermatoxic effects of gossypol on the structural organization of the sperm midpiece in late spermatogenesis. Gossypol appears to cause damage to the mitochondrial sheath of cells during the latter phases of spermatogenesis, with lesions being first detected in elongating spermatids. So, the types of lesion first observed at extragonadal sites suggested structural failure in already weakened structures, possibly exacerbated by the onset of sperm motility.

In ultrastructural studies, segmental aplasia of the mitochondrial sheath has been consistently identified with gossypol treatment in rats, monkeys, rabbits and bulls (OKO; HRUDKA, 1982; 1984b; CHENOWETH et al., 2000). This was considered to be pathognomonic for gossypol spermatoxicity (OKO; HRUDKA, 1982), which allows it to be differentiated from other causes of sperm axonemal disruption. In the present study, there was a linear trend between gossypol level in diet and the segmental aplasia lesion in sperm. The WCS and CSC group showed higher level of SAMS than others diets, which showed that high levels of gossypol in the diet led to higher occurrence of this lesion. However, we disagree that this lesion is pathognomonic for gossypol spermatoxicity, because we found it in spermatozoa from the CTL group (Figure 4.5). Others authors also have occasionally observed this lesion in bulls that not received cotton products (BURGESS; CHENOWETH, 1975).

In conclusion, the CTL group had the lowest total sperm defects and better seminiferous epithelium structure. When compared to the CTL group, CSM had more isolated head defect, WCS and CSM had more coiled tail defect and CSC had more proximal cytoplasmic droplets. Animals from the CSC group had lower testosterone concentration, and WCS and CSC had lower SIS and higher number of SAMS. Therefore, overall reproductive status of CTL group was better than the others, consequently, the cotton co-products had a negative impact on it. However, the increase in gossypol concentration in the diet did not demonstrate a proportional increase in injuries, which probably was related to the different

feedstuffs (gossypol sources). Thus, differences in rumen function between feedstuffs can affect free gossypol bioavailability (CHASE et al., 1994).

In ruminants, there are difficulties in controlling the amount of bioavailable gossypol. In studies where total gossypol levels in plasma were checked and adverse effects were found, the levels varied from 26.2 to 73.0 µg/g in treated bulls and no gossypol was detected in the plasma of control bulls (CHENOWETH et al., 1994). In contrast, in a study with bulls where no qualitative or quantitative semen changes were detected following the feeding of cottonseed meal for 132 days, no gossypol was detected in the plasma or in the other tissues of the treated bulls (JIMENEZ et al., 1989). Moreover, there is a variation between individuals. In studies with humans, 15% of mens failed to suppress spermatogenesis, although had similar plasma gossypol levels than the others which had sperm suppression (COUTINHO, 2002).

4.5. Conclusions

The diets did not alter lamb growth. However, the cotton co-products have a negative impact on reproductive system of puberal lambs regardless gossypol concentration. Control group had the lowest total sperm defects and better seminiferous epithelium structure. Also, control had higher testosterone concentration than high-oil cottonseed meal, and the occurrence of segmental aplasia of mitochondrial sheath increased according to higher levels of gossypol in the diet.

References

BILLIG, H. et al. Apoptosis in testis germ-cells - developmental-changes in gonadotropin dependence and localization to selective tubule stages. **Endocrinology**, Springfield, v. 136, n. 1, p. 5-12, 1995. ISSN 0013-7227.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Brasil projeções do agronegócio 2011/2012 a 2021/2022**. Brasília, DF, 2012. 50 p.

BURGESS, G. W.; CHENOWETH, P. J. Mid-piece abnormalities in bovine semen following experimental and natural cases of bovine ephemeral fever. **The British Veterinary Journal**, London, v. 131, n. 5, p. 536-44, 1975 1975. ISSN 0007-1935. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/1192163 >.

CHASE, C. et al. Growth and reproductive development in brahman bulls fed diets containing gossypol. **Journal of Animal Science**, Albany, v. 72, n. 2, p. 445-452, 1994. ISSN 0021-8812.

CHENOWETH, P. et al. Characterization of gossypol-induced sperm abnormalities in bulls. **Theriogenology**, New York, v. 53, n. 5, p. 1193-1203, 2000. ISSN 0093-691X.

_____. Effects of dietary gossypol on aspects of semen quality, sperm morphology and sperm production in young brahman bulls. **Theriogenology**, New York, v. 42, n. 1, p. 1-13, 1994. ISSN 0093-691X.

COUTINHO, E. M. Gossypol: a contraceptive for men. **Contraception**, Los Altos, v. 65, n. 4, p. 259-263, 2002. ISSN 0010-7824.

CUNHA, M. D. G. G. et al. Effect of diets containing whole cottonseed on the quality of sheep semen. **Acta Scientiarum. Animal Sciences**, Maringá, v. 34, p. 305-311, 2012. ISSN 1807-8672.

DABROWSKI, K. et al. Effects of diets containing gossypol on reproductive capacity of rainbow trout (Oncorhynchus mykiss). **Biology of Reproduction**, Champaign, v. 62, n. 2, p. 227-34, 2000. ISSN 0006-3363.

DE ANDRADE, S. F. et al. Epididymis-specific pathologic disorders in rats exposed to gossypol from weaning through puberty. **Toxicologic Pathology**, Lawrence, v. 34, n. 6, p. 730-737, 2006. ISSN 0192-6233.

DE PEYSTER, A.; SREBNIK, H. H. Reproductive endocrine function in gossypol-treated male rats. **International Journal of Fertility**, New York, v. 33, n. 5, p. 362-371, 1988. ISSN 0020-725X.

EL-SHARAKY, A. et al. Spermatotoxicity, biochemical changes and histological alteration induced by gossypol in testicular and hepatic tissues of male rats. **Food and Chemical Toxicology**, London, v. 48, n. 12, p. 3354-3361, 2010. ISSN 0278-6915.

GEIGER, P. Radioimmunoassay data handling and calculations with a graphics statistics computer-program. **Biochemical Medicine and Metabolic Biology**, Amsterdam, v. 48, n. 1, p. 74-80, 1992. ISSN 0885-4505.

GIZEJEWSKI, Z. et al. Cottonseed feeding delivers sufficient quantities of gossypol as a male deer contraceptive. **European Journal of Wildlife Research**, Heidelberg, v. 54, n. 3, p. 469-477, 2008. ISSN 1612-4642.

HOFFER, A. Effects of gossypol on the seminiferous epithelium in the rat - a light and electron-microscope study. **Biology of Reproduction**, Champaign, v. 28, n. 4, p. 1007-1020, 1983. ISSN 0006-3363.

HOFFER, A. et al. Ultrastructural, fertility, and spermicidal studies with isomers and derivatives of gossypol in male hamsters. **Biology of Reproduction**, Champaign, v. 37, n. 4, p. 909-924, 1987. ISSN 0006-3363.

JIMENEZ, D. A. et al. Effect of feeding gossypol in cottonseed meal on growth, semen quality, and spermatogenesis of yearling Holstein bulls. **Journal of Dairy Science**, Lancaster, v. 72, n. 7, p. 1866-75, 1989. ISSN 0022-0302.

LARSEN, R.; CHENOWETH, P. Diadem crater defects in spermatozoa from 2 related angus bulls. **Molecular Reproduction and Development**, Hoboken, v. 25, n. 1, p. 87-96, 1990. ISSN 1040-452X.

MOH, P. P. et al. Effect of gossypol on 5-alpha-reductase and 3-mu-hydroxysteroid dehydrogenase-activities in adult-rat testes. **Research Communications in Chemical Pathology and Pharmacology**, New York, v. 82, n. 1, p. 12-26, 1993. ISSN 0034-5164.

OKO, R.; HRUDKA, F. Segmental aplasia of the mitochondrial sheath and sequelae induced by gossypol in rat spermatozoa. **Biology of Reproduction**, Champaing, v. 26, n. 1, p. 183-195, 1982. ISSN 0006-3363.

_____. Comparison of the effects of gossypol, estradiol-17-beta and testosterone compensation on male-rat reproductive-organs. **Biology of Reproduction**, Champaing, v. 30, n. 5, p. 1198-1207, 1984a. ISSN 0006-3363.

_____. Gossypol-induced early and delayed-effects in the seminiferous epithelium of the adult-rat. **Contraceptive Delivery Systems**, Lancaster, v. 5, n. 4, p. 335-356, 1984 1984b. ISSN 0143-6112.

RANDEL, R.; CHASE, C.; WYSE, S. Effects of gossypol and cottonseed products on reproduction of mammals. **Journal of Animal Science**, Albany, v. 70, n. 5, p. 1628-1638, 1992. ISSN 0021-8812.

REISER, R.; FU, H. Mechanism of gossypol detoxification by ruminant animals. **Journal of Nutrition**, Bethesda, v. 76, n. 2, p. 215-218, 1962. ISSN 0022-3166.

NRC. National Research Council. **Nutrient requirements of small ruminants:** sheep, goats, cervids, and new world camelids. Washington, DC: The National Academies Press, 2007. ISBN 9780309102131. Disponível em: < http://www.nap.edu/openbook.php?record_id=11654 >.

SAITO, K. et al. Spermiation failure is a major contributor to early spermatogenic suppression caused by hormone withdrawal in adult rats. **Endocrinology**, Springfield, v. 141, n. 8, p. 2779-2785, 2000. ISSN 0013-7227.

SHANDILYA, L. et al. Effects of gossypol on reproductive and endocrine functions of male cynomolgus monkeys (Macaca fascicularis). **Biology of Reproduction**, Champaing, v. 27, n. 1, p. 241-52, 1982. ISSN 0006-3363.

SOUFIR, J. C. et al. Gossypol-induced modifications in the microenvironment of rat epididymal spermatozoa. **Journal of Reproduction and Fertility**, Oxford, v. 86, n. 2, p. 427-434, 1989. ISSN 0022-4251.

STEGER, K.; WROBEL, K. Postnatal development of ovine seminiferous tubules: An electron microscopical and morphometric study. **Annals of Anatomy-Anatomischer Anzeiger**, Jena, v. 178, n. 3, p. 201-213, 1996. ISSN 0940-9602.

UDOH, P.; PATIL, D.; DESHPANDE, M. Histopathological and biochemical effects of gossypol acetate on pituitary-gonadal axis of male albino-rats. **Contraception**, Los Altos, v. 45, n. 5, p. 493-509, 1992. ISSN 0010-7824.

WANG, J. M. et al. Effect of gossypol on testicular blood flow and testosterone production in rats. **Journal of Reproduction and Fertility**, Oxford, v. 71, n. 1, p. 127-33, maio 1984. ISSN 0022-4251.

WANG, M. Analysis of gossypol by high-performance liquid-chromatography. **Journal of Ethnopharmacology**, Lausanne, v. 20, n. 1, p. 1-11, 1987. ISSN 0378-8741.

WROBEL, K.; REICHOLD, J.; SCHIMMEL, M. Quantitative morphology of the ovine seminiferous epithelium. **Annals of Anatomy-Anatomischer Anzeiger**, Jena, v. 177, n. 1, p. 19-32, 1995. ISSN 0940-9602.

5. FATTY ACIDS PROFILE OF MEAT FROM LAMBS FED WITH COTTONSEED CO-PRODUCTS

Abstract

We evaluated the changes on carcass traits and fatty acid profile of meat induced by feeding lambs with cottonseed co-products. Twenty-four 5-months old ram lambs received one of four diets: 20% of dry matter intake (DMI) of whole cottonseed (WCS), 20%DMI cottonseed meal (CSM), 20%DMI high oil cottonseed meal (CSC) and a control group (CTL) without cottonseed co-products. After 95 experimental days, the lambs were slaughtered. Carcass weight and 12th rib composition (chemical and centesimal) were measured. Meat samples of *Longissumus dorsi* muscle were taken for fatty acid profile analysis. The animals that received CSM showed higher hot carcass weight, carcass yield and rib eye area than animals from the WCS group. Meat from CSM and CSC groups had higher levels of conjugated linolenic acid (CLA) than others and yet CSC group showed higher vaccenic acid than others. Meat from animals that received whole cottonseed had less unsaturated fatty acids, CLA and vaccenic acid. Therefore, processed cottonseed co-products (CSM and CSC) must be preferred for use in ruminant feed rather than whole cottonseed. The meat from animals that did not receive cotton co-products had higher n-3 fatty acid, and also better n-6 to n-3 ratio compared to others, which can be a problem to use of these products in ruminant nutrition due to current great importance given to these fatty acids in human nutrition.

Keywords: Whole cottonseed. Cottonseed meal. Carcass traits. Conjugated linolenic acid. Vaccenic acid.

5.1. Introduction

One of the important issues in food industry is the development of functional foods that enhance food safety, human nutrition, and health. Given the increasing prevalence of obesity and cardiovascular disease in developed nations, changes in product composition could contribute to improvements in consumer health. A decrease in the prevalence of deleterious fats and cholesterol as well as an increase in the prevalence of monounsaturated and n-3 fatty acids are consistent with dietary recommendations for cardiovascular health (SIMOPOULOS, 1999). Diets with a high n-6 to n-3 ratio may contribute to the prevalence of many diseases, such as coronary artery disease, cancer, diabetes, arthritis and depression. Studies indicated that a high intake of n-6 fatty acids shifts the physiological state to one that is prothrombotic and proaggregatory, while n-3 fatty acids have anti-inflammatory, antithrombotic, antiarrhythmic, hypolipidemic and vasodilatory properties (SIMOPOULOS, 2002).

Industrial production of animal feeds rich in grains containing n-6 fatty acids, has led to production of meat and milk rich in n-6 fatty acids and poor in n-3 fatty acids (CRAWFORD et al., 1970). Recently, there is increasing discussions in terms of human health about a group of specific fatty acids (conjugated linoleic acids: CLA) that occur in ruminant products. Conjugated linoleic acid, principally the C18:2 *cis*9 *trans*11 isomer, may be anticarcinogenic and anti-atherosclerotic, decrease fat accumulation, and can modulate the immune response and thus enhance cell-mediated responses, and decrease the inflammatory response (PARIZA; PARK; COOK, 2001).

At present, changes in fatty acid profile of food from animal sources is becoming an important research issue (RAES; DE SMET; DEMEYER, 2004; WOOD et al., 2004; DALEY et al., 2010).These changes can be promoted through animal nutrition or/and animal genetics (DE SMET; RAES; DEMEYER, 2004). Some studies also exist on the production of transgenic animals that produce linoleic acid (C18:2n-6) and linolenic acid (C18:3n-3) (SAEKI et al., 2004); and genetically modified pigs were developed to express an n-3 fatty acid desaturase capable of converting n-6 fatty acids to n-3 fatty acids (LAI et al., 2006). The fatty acid profile of diet is direct related to the profile of meat, so there are a large number of studies on animal nutrition aiming to produce functional foods (DE SMET; RAES; DEMEYER, 2004; RAES; DE SMET; DEMEYER, 2004). For instance, increased n-3

content in meat can be achieved by including fish oil/meal, linseed and/or forages in animal diet (DALEY et al., 2010; OLIVEIRA et al., 2011).

On the other hand, low cost alternatives to feed ruminants are sought, aiming to produce low cost food. Whole cottonseed and its co-products are alternative feed sources, which can decrease the cost of the animal diet. These products have high levels of fatty acids, which may lead to increased weight gain, greater fat deposition on the carcass, and some changes in fatty acid profile of meat, which can influence its acceptability by consumer and impact in human health (MADRUGA et al., 2008).

A major limitation for using cotton co-products in animal nutrition is the presence of high levels of gossypol. This is a highly reactive substance, capable of acting as a phenolic and as an aldehydic compound. The phenolic groups of gossypol react readily to form esters and ethers; the aldehyde groups react with amines to form Schiff's bases, and with organic acids to form heat labile compounds (EL-SHARAKY et al., 2010). Gossypol toxicity is generally low in ruminants due to rumen environment that promotes binding of gossypol to proteins, which reduce the absorption and turns it physiologically inactive. But, as gossypol can impair the activity of some enzymes (MOH et al., 1993; EL-SHARAKY et al., 2010), some questions on the effect of gossypol on ruminant metabolism and, consequently, on meat quality are unknown, as well as the possibility of residues of this compound in meat.

In this study we investigated the impact on fatty acid profile of meat, carcass traits and meat composition of feeding lambs with cotton co-products. Moreover, aiming to explain the differences between feedstuffs, we verified the gossypol effect on the traits evaluated and the relation between fatty acid profiles of diet and meat.

5.2. Material and Methods

Twenty-four Santa Inês ram lambs, 5-months old, with mean body weight equal to 20.6 ± 1.9 kg were housed in individual covered pens on a concrete floor. These animals were divided randomly in four diets: control (without cottonseed co-products, CTL); whole cottonseed (WCS); solvent-extracted cottonseed meal (CSM); pressure-extracted high oil cottonseed meal (CSC) (as described previously in Section 4.2 and Table 4.1). The experimental period lasted 95 days and was preceded by an adaptation period of 14 days.

At the end of experimental period, the animals were weighed (SBW) and slaughtered after a 24 hour fasting, according to Brazilian laws. Hot carcass weight (HCW) was taken. Carcass yield (Y) of hot carcass was calculated (Y=HCW/SBW). After 24 hours at 0 °C, cold

carcass weight was taken (CCW) and a fraction of the rib was removed by transverse cuts at the 11^{th} and 13^{th} ribs. Rib eye area was determined using a standard transparent grade (0.5 cm^2/cell), by drawing around the *Longissimus dorsi* muscle exposed by the transverse cut of the 12^{th} intercostal space. The 12^{th} rib was weighed and tissues were dissected into their compositions (muscle, bone and fat). The component rib tissues were minced together for freeze drying. Muscle samples and the rib tissues were analyzed using AOAC (1995) procedures.

For fatty acid profile analysis, lipids were extracted from the muscle and diets in accordance with procedures established by Folch, Lees and Stanley (1957) and were methylated according to methods described by Hara and Radin (1978). The transmethylated samples were analyzed with a gas chromatograph (model Focus CG-Finnigan, Thermo Finnigan, San Jose, CA) with a flame-ionization detector and a capillary column (CP-Sil 88; Varian, Palo Alto, CA) measuring 100 m in length × 0.25 mm i.d., with a thickness of 0.20 μm (Supelco, Bellefonte, PA). Hydrogen was used as the carrier gas at a flow rate of 1.8 mL/min. The initial temperature of the oven was 70°C and was increased by 13°C/min to 175°C, where it was maintained for 27 min. The temperature was then increased by 4°C/min to 215°C, where it was maintained for 9 min, followed by another increase by 7°C/min to 230°C, where it remained for 5 min. The temperature of the injector was 250°C, and the temperature of the detector was 300°C. Identification of the fatty acids was done by comparison of the retention times with standards of fatty acids from butter, and the percentage of fatty acids was obtained by means of Chromquest 4.1 software (Thermo Electron, Milan, Italy). The fatty acid profiles of diets are shown in Table 5.1.

Table 5.1 - Fatty acids profile (g/100g of total fatty acids) of four experimental concentrates: whole cottonseed (WCS), cottonseed meal (CSM), high oil cottonseed meal (CSC), and a control group without cotton seed (CTL)

	CTL	**WCS**	**CSC**	**CSM**
C6:0	0.14	0.06	0.50	0.17
C14:0	0.14	0.68	0.49	0.21
C16:0	13.88	23.35	22.31	15.51
C16:1 c9	0.14	0.43	0.26	0.17
C17:0	0.13	0.12	0.17	0.12
C18:0	3.08	2.56	3.78	3.14
C18:1 c9	25.69	17.13	24.54	25.70
C18:1 c11	1.58	1.05	1.70	1.71
C18:1 c12	0.64	0.41	0.68	0.59
C18:1 c13	0.20	0.14	0.23	0.25
C18:2 t11 c15	0.08	0.04	0.12	0.12
C18:2 c9 c12	49.25	52.97	41.42	47.44
C18:3 n6	0.21	0.15	0.25	0.30
C20:0	0.11	0.01	0.13	0.23
C18:3 n3	3.76	0.29	2.41	3.36
C20:1	0.05	0.04	0.09	0.18
C21:0	0.00	0.00	0.16	0.06
C22:0	0.25	0.12	0.21	0.24
C24:0	0.13	0.08	0.09	0.12
SFA	17.99	27.11	28.03	19.89
UFA	81.76	72.74	71.97	79.92
MUFA	28.41	19.29	27.61	28.70
PUFA	53.35	53.45	44.37	51.22
n-6	49.47	53.12	41.67	47.73
n-3	3.76	0.29	2.50	3.36
n-6:n-3	13.17	183.79	16.68	14.19
UFA:SFA	4.55	2.68	2.57	4.02
PUFA:SFA	2.97	1.97	1.58	2.58

Showed only fatty acids with results greater than 0.1 g/100g of fat. Control: without cottonseed co-products in concentrate; WCS: 40% of whole cottonseed in concentrate; CSM: 40% of cottonseed meal in concentrate; CSC: 40% of high oil cottonseed meal in concentrate.

The amount of desirable fatty acids (DFA) was determined as MUFA, PUFA and stearic acid according to Landim et al. (2011). The activities of the Δ^9-desaturase (C16 and C18) and elongase enzymes were determined as described by De Smet, Raes and Demeyer (2004), using mathematical indices. The atherogenicity index (ATHERO) was calculated in accordance with the method of Ulbricht and Southgate (1991). This index is considered an indicator for the risk of cardiovascular disease. Calculations were performed as follows:

DFA = MUFA + PUFA + C18:0 ……..…….....(1)

Δ^9-desaturase C16 = 100[(C16:1 *cis*-9)/ (C16:1 *cis*-9 + C16:0)] .....(2)

Δ^9-desaturase C18 = 100[(C18:1 *cis*-9)/(C18:1 *cis*-9 + C18:0)] .....(3)

Elongase = 100[(C18:0 + C18:1 *cis*-9)/ (C16:0 + C16:1 *cis*-9 + C18:0 + C18:1 *cis*-9)](4)

ATHERO = [C12:0 + 4*(C14:0) + C16:0]/(ΣSFA + ΣPUFA) ……...…….(5)

Carcass traits, 12th rib composition, and meat composition data was submitted to analysis of variance using the GLM procedure in SAS®. The fatty acid profile was analyzed using GLM procedure to verify effect of treatments. The effect of ether extract in diet, free gossypol level in diet and ether extract in meat was determined by a stepwise multiple regression to each fatty acid in the meat. The relation between fatty acid profile, gossypol level, ether extract in diet and ether extract in meat was analyzed through factor and correlation analyses in SAS®. When the factors explained less than 0.50 of variance of the variable, this was excluded. Aiming to explain the differences found between treatments, we carried out a stepwise multiple regression analysis between fatty acid profiles in diet and meat to verify if some fatty acid in diet were direct related to other in meat. In all analyzes, means were compared through least square means when $p<0.05$, using pdiff statement in SAS®. And the stepwise multiple regressions used 0.05 as significant level to independent variable stay in the model.

5.3. Results

The estimated free gossypol intake per treatment was 0, 16.32, 6.98 and 5.47 mg/kg of Body Weight (BW) for CTL, WCS, CSC and CSM, respectively. For carcass traits, the WCS group had lower HCW, CCW and Rib eye area than CSM group and lower Y than CTL group (Table 5.2). The 12th rib composition showed that the CSC group had lower bone (mass and proportion) than CTL and consequently the muscle proportion in CSC group was higher than CTL (Table 5.3). The groups did not differ in fat content and ether extract of 12th rib. The meat composition (*Longissimus dorsi* muscle) also did not differ between groups (Table 5.4).

Table 5.2 - Least square means of carcass traits of Santa Ines lambs fed with whole cottonseed (WCS), cottonseed meal (CSM), high oil cottonseed meal (CSC), and a control group without cotton seed (CTL), during 95 days before slaughter

	CTL	**WCS**	**CSC**	**CSM**	**Mean**	**CV (%)**
SBW (kg)	29.45	29.80	30.46	31.37	30.26	5.27
HCW (kg)	13.80^{ab}	13.05^{b}	13.58^{ab}	14.42^{a}	13.72	8.09
CCW (kg)	13.50^{ab}	12.75^{b}	13.32^{ab}	14.08^{a}	13.42	8.09
Carcass Yield (%)	46.7^{a}	43.8^{b}	44.6^{ab}	46.0^{ab}	45.3	4.74
Rib eye area (cm²)	11.10^{ab}	9.00^{b}	10.05^{ab}	11.96^{a}	10.76	16.22

Different letters in the same row indicate statistical difference ($P < 0.05$). CTL: without cottonseed co-products in diet; WCS: 20% of whole cottonseed in diet; CSM: 20% of cottonseed meal in diet; CSC: 20% of high oil cottonseed meal in diet. CV: coefficient of variation (%), SBW: Shrunk Body Weight (kg), HCW: Hot Carcass Weight (kg), CCW: Cold Carcass Weight (kg), Carcass Yield = HCW/SBW.

Table 5.3 - Least square means of muscle, fat and bone from 12th rib (dissection) from lambs fed with whole cottonseed (WCS), cottonseed meal (CSM), high oil cottonseed meal (CSC), and a control group without cotton seed (CTL), during 95 days before slaughter

	CTL	WCS	CSC	CSM	Mean	CV (%)
Muscle (g)	53.99	49.21	55.04	59.62	54.44	25.19
Fat (g)	22.47	21.71	17.83	21.46	21.00	31.55
Bone (g)	27.07^{a}	21.25^{ab}	18.13^{b}	24.77^{ab}	23.00	26.37
Total Weight (g)	103.53	92.16	91.01	105.85	98.45	22.90
Muscle (%)	52.13^{b}	53.31^{b}	59.77^{a}	56.56^{ab}	55.25	7.94
Fat (%)	21.95	22.93	19.95	19.98	21.25	17.76
Bone (%)	25.93^{a}	23.76^{ab}	20.28^{b}	23.46^{ab}	23.49	18.38
Muscle:Fat ratio	2.43	2.37	3.11	2.99	2.71	23.84
Muscle:Bone ratio	2.07	2.41	3.09	2.47	2.48	28.94
DM (%)	45.14^{a}	41.43^{ab}	39.81^{b}	41.39^{ab}	42.03	9.90
CP (%)	43.80^{b}	49.74^{a}	51.79^{a}	48.13^{ab}	48.22	9.93
EE (%)	42.83	37.92	37.93	35.76	38.64	19.82
A (%)	14.53	16.89	12.38	15.35	14.89	38.33

Different letters in the same row indicate statistical difference (P<0.05); CTL: without cottonseed co-products in diet; WCS: 20% of whole cottonseed in diet; CSM: 20% of cottonseed meal in diet; CSC: 20% of high oil cottonseed meal in diet; CV: coefficient of variation (%); DM: Dry matter, CP: Crude Protein, EE: Ether extract, A: ash content; obtained by bromatological analysis using Association of Official Agricultural Chemistry (AOAC, 1995) procedures. Results of CP, EE and A are in DM basis.

Table 5.4 - Least square means of *Longissimus dorsi* muscle composition at 12th rib from lambs fed with whole cottonseed (WCS), cottonseed meal (CSM), high oil cottonseed meal (CSC), and a control group without cotton seed (CTL), during 95 days before slaughter

	CTL	WCS	CSC	CSM	Mean	CV (%)
DM (%)	27.86	28.00	27.54	27.50	27.73	5.77
CP (%)	77.37	79.91	77.18	77.09	77.92	8.06
EE (%)	28.40	25.59	29.12	29.14	28.02	21.63
A (%)	3.15	3.39	3.23	3.52	3.33	13.73

CTL: without cottonseed co-products in diet; WCS: 20% of whole cottonseed in diet; CSM: 20% of cottonseed meal in diet; CSC: 20% of high oil cottonseed meal in diet; CV: coefficient of variation (%); DM: Dry matter (%), CP: Crude Protein in dry matter (%), EE: Ether extract in dry matter (%), A: ash content in dry matter (%); obtained by bromatological analysis using Association of Official Agricultural Chemistry (AOAC, 1995) procedures.

Fatty acid profile analysis showed that meat from lambs fed with whole cottonseed had higher saturated fatty acids (SFA), C18:0 and, consequently, lower unsaturated to saturated ratio (UFA:SFA) than others groups (Tables 5.5 and 5.6). Moreover, meat from WCS group had the lowest level of conjugated linoleic acid (C18:2c9t11), vaccenic acid (C18:1t11) and C18:3n-3. The meat from WCS group also showed lower C20:5n-3 and C22:6n-3 than CTL group. The desirable fatty acids (DFA) in meat from CTL group were higher than WCS group (Table 5.6). Meat from CSM and CSC groups showed higher level of conjugated linoleic acid (CLA – C18:2c9t11) compared to others groups. And the CSC group had the highest vaccenic acid (C18:1t11) level. Meat from CTL group showed more n-3 fatty acids and lower n-6 to n-3 ratio than others.

The regression between gossypol concentrations in diets (Gos) (linear, quadratic and cubic effects) and fatty acids in meat are showed in Table 5.7. In general, gossypol level had a positive relationship with C15:0*iso*, C16:0, C17:0, C18:0 and SFA. Gossypol level had a negative relationship with C18:3n3, C18:1 c13, n3 total, UFA and UFA:SFA. As the ether extract in diet and in muscle may influence the fatty acid profile in meat, we realized a multiple regression between these variables. However, little modifications in gossypol effect described previously were seen, hence these regressions are shown in Appendix A.

Table 5.5 - Least square means of fatty acids profile (g/100g of total fatty acids) of meat from Santa Inês lambs fed whole cottonseed (WCS), cottonseed meal (CSM), high oil cottonseed meal (CSC), and a control group without cotton seed (CTL), during 95 days before slaughter

	CTL	WCS	CSC	CSM	Mean	CV (%)
C10:0	0.119	0.109	0.113	0.119	0.115	25.57
C12:0	0.081	0.069	0.081	0.082	0.078	16.93
C13:0	0.149	0.119	0.098	0.139	0.128	48.41
C14:0	1.475	1.568	1.691	1.787	1.628	26.85
C15:0 *iso*	0.088^{b}	0.128^{a}	0.111^{ab}	0.093^{b}	0.105	24.54
C15:0 *anteiso*	0.936	0.948	0.965	1.199	1.014	34.53
C14:1 c9	0.281	0.256	0.283	0.325	0.286	34.15
C15:0	0.524	0.492	0.473	0.516	0.502	20.43
C16:0 *iso*	0.130^{a}	0.119^{ab}	0.088^{b}	0.098^{ab}	0.109	26.55
C16:0	19.32^{b}	21.67^{a}	19.71^{b}	19.92^{ab}	20.175	7.48
C17:0 *iso*	0.067	0.052	0.067	0.068	0.063	29.38
C16:1 c9	0.865	0.912	0.861	0.980	0.906	19.89
C17:0	0.625^{ab}	0.697^{a}	0.563^{b}	0.577^{b}	0.618	10.23
C17:1	0.365^{ab}	0.364^{ab}	0.290^{b}	0.372^{a}	0.350	16.72
C18:0	18.88^{b}	23.27^{a}	19.29^{b}	18.16^{b}	19.928	12.62
C18:1 t6-t7-t8-t9	0.619^{b}	0.344^{c}	0.885^{a}	0.646^{b}	0.612	24.08
C18:1 t10-t11-t12	3.371^{b}	1.122^{c}	5.393^{a}	3.775^{b}	3.329	24.76
C18:1 c9	29.02	30.72	27.38	30.12	29.395	11.47
C18:1 c11	1.707	1.803	1.523	1.652	1.678	20.48
C18:1 c12	0.636	0.614	0.582	0.559	0.599	25.02
C18:1 c13	0.743^{b}	0.396^{c}	1.221^{a}	0.559^{bc}	0.708	22.62
C18:1 t16	0.208^{b}	0.194^{b}	0.295^{a}	0.225^{b}	0.228	15.42
C18:2 t11c15	0.130	0.114	0.189	0.195	0.155	46.86
C18:2 c9c12n6	9.384	6.624	8.664	8.062	8.163	37.04
C18:3n6	0.070	0.055	0.064	0.058	0.062	31.13
C20:0	0.116^{a}	0.077^{b}	0.109^{a}	0.095^{ab}	0.099	24.28
C18:3n3	0.358^{a}	0.161^{c}	0.263^{b}	0.279^{ab}	0.265	25.18
C18:2 c9t11	0.760^{b}	0.285^{c}	1.262^{a}	1.022^{a}	0.813	24.15
C24:0	0.106	0.237	0.228	0.227	0.198	57.38
C20:3n6	0.240	0.168	0.170	0.158	0.184	51.52
C20:4n6	4.069	2.706	2.759	3.300	3.228	56.19
C20:5n3	0.164^{a}	0.069^{b}	0.110^{ab}	0.085^{ab}	0.107	68.57
C22:5n3	0.444	0.263	0.311	0.312	0.333	44.32
C22:6n3	0.081^{a}	0.029^{b}	0.027^{b}	0.041^{ab}	0.045	72.69

Showed only fatty acids with least square means greater than 0.05 g/100g of fat. Different letters in the same row indicate statistical difference ($P < 0.05$); CTL: without cottonseed co-products in diet; WCS: 20% of whole cottonseed in diet; CSM: 20% of cottonseed meal in diet; CSC: 20% of high oil cottonseed meal in diet; CV: coefficient of variation (%).

Table 5.6 - Least square means of overall fatty acids, ratios and indices of meat from Santa Ines lambs fed with whole cottonseed (WCS), cottonseed meal (CSM), high oil cottonseed meal (CSC), and a control group without cotton seed (CTL), during 95 days before slaughter

	CTL	WCS	CSC	CSM	Mean	CV (%)
SFA	42.68^{b}	49.64^{a}	43.63^{b}	43.13^{b}	44.82	7.36
UFA	53.63^{a}	47.27^{b}	52.62^{a}	52.80^{a}	51.54	5.71
MUFA	37.90	36.78	38.77	39.26	38.15	10.17
PUFA	15.74	10.49	13.85	13.54	13.38	37.95
n-6	13.76	9.55	11.66	11.58	11.64	42.10
n-3	1.05^{a}	0.52^{b}	0.71^{b}	0.72^{b}	0.75	41.64
n6:n3	12.85^{b}	17.93^{a}	16.39^{a}	16.20^{a}	15.82	11.93
UFA:SFA	1.26^{a}	0.97^{b}	1.21^{a}	1.23^{a}	1.16	11.88
PUFA:SFA	0.37	0.22	0.32	0.32	0.31	45.06
DFA	72.51^{a}	70.54^{b}	71.91^{ab}	70.96^{ab}	71.46	1.78
D9C16	4.20	4.04	4.15	4.65	4.27	16.81
D9C18	60.22^{ab}	57.03^{b}	58.69^{ab}	62.35^{a}	59.61	7.24
ELONGASE	70.14	70.36	69.31	69.70	69.91	1.85
ATHERO	0.45	0.47	0.47	0.48	0.47	14.79

Different letters in the same row indicate statistical difference (P<0.05 CTL: without cottonseed co-products in diet; WCS: 20% of whole cottonseed in diet; CSM: 20% of cottonseed meal in diet; CSC: 20% of high oil cottonseed meal in diet; CV: coefficient of variation (%); SFA: Saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n6: n-6 fatty acids; n3: n-3 fatty acids; n6:n3: n-6 divided by n-3; UFA:SFA: unsaturated divided by saturated fatty acids; PUFA:SFA: polyunsaturated divided by saturated fatty acids; DFA: desirable fatty acids; D9C16, D9C18, ELONGASE: Δ^{9}-desaturase Cis-9 C16 activity, Δ^{9}-desaturase Cis-9 C18 activity, elongase enzyme activity, respectively, determined as described by Oliveira et al. (2011) using mathematical indices; ATHERO: atherogenicity index calculated in accordance with the method of Ulbricht and Southgate (1991).

The correlations (r) obtained supported the positive relationship between gossypol in diet and saturated fatty acids (r = 0.54 to C16:0; 0.44 to C17:0; 0.61 to C18:0 and 0.67 to SFA). The correlations also showed a negative relationship between gossypol in diet and n-3 fatty acids (r = -0.81 to C18:3n3; -0.48 to C20:5n3; -0.46 to C22:5n3; -0.51 to C22:6n3 and -0.60 to n3 total). The gossypol concentration in diet also showed a negative correlation with PUFA (-0.43) and DFA (-0.47).

Table 5.7 - Regression equations (testing linear, quadratic and cubic effect) between gossypol level in diet (Gos) and each fatty acid of meat from lambs fed with cottonseed co-products

Fatty acids	Regression	R^2	*P*
C15:0 *iso*	= 0.029 + 0.063*Gos	0.30	0.0067
C16:0	= 0.456 + 2.647*Gos²	0.30	0.0066
C17:0	= 0.077 + 6.331*Gos³	0.36	0.0025
C18:0	= 0.445 + 54.196*Gos³	0.44	0.0006
C18:1 t6-t7-t8-t9	= 0.071 + 13.55*Gos² – 149.34*Gos³	0.61	0.0175
C18:1 t10-t11-t12	= 0.182 – 6.26*Gos + 260.49*Gos² - 2025.32*Gos³	0.83	0.0441
C18:1 c13	= 0.0897 – 24.63*Gos³	0.35	0.0028
C18:3 n3	= 0.059 – 0.191*Gos	0.66	0.0001
C18:2 c9 t11	= 0.086 + 24.67*Gos² - 272.59*Gos³	0.84	0.0002
C22:6 n3	= 0.028 – 0.27*Gos + 15.97*Gos³	0.41	0.0362
SFA	= 0.71 + 6.941*Gos²	0.49	0.0002
UFA	= 0.822 – 6.156*Gos²	0.49	0.0002
n-3	= 0.098 – 0.274*Gos	0.36	0.0023
UFA:SFA	= 0.113 – 1.391*Gos²	0.49	0.0002

Showed only regressions with $R^2 > 0.3$. R^2: determination coefficient; *P*: p-value. SFA: Saturated fatty acids; UFA: unsaturated fatty acids; n3: n-3 fatty acids; UFA:SFA: unsaturated divided by saturated fatty acids; PUFA:SFA: polyunsaturated divided by saturated fatty acids.

The two first factors explained 77.1% of variance between variables (Figure 5.1). The first factor demonstrated PUFA, C18:2c9c12, n6 and n3 fatty acids on one side and, on the other side, MUFA, ATHERO, n6:n3, C18:1c9, SFA, C14:0, C16:0 and C18:0, so when the proportions of the first group increase the second group decrease and vice-versa. The second factor separated gossypol level in diet together with SFA, and at opposition to CLA (C18:2c9t11) and vaccenic acid (C18:1t10-12), which demonstrated that higher gossypol concentration in diets was related to lower CLA and vaccenic acid levels in meat.

The regressions between each fatty acid in meat and fatty acid profile of experimental diets indicated that: an increase in C8:0 content in the diet led to higher C18:1t10-t11-t12 in meat (C18:1t10-t11-t12 = -0.795 + 147.754*C8:0d; $R^2 = 0.80$); C18:2c9t11 (CLA) content in meat was positively related to C8:0 and negatively to C22:2 in the diet (C18:2c9t11 = -0.294 + 44.099*C8:0d – 14.579*C22:2d; $R^2 = 0.81$). The C17:0 *iso* in the diet showed a negative relationship with C18:3n3, C20:5n3, n-3 total and DFA in meat. Moreover, C18:1c9 in diet

was determinant for SFA (negative effect) and UFA (positive effect) content in meat. The others regressions are available in table 5.8.

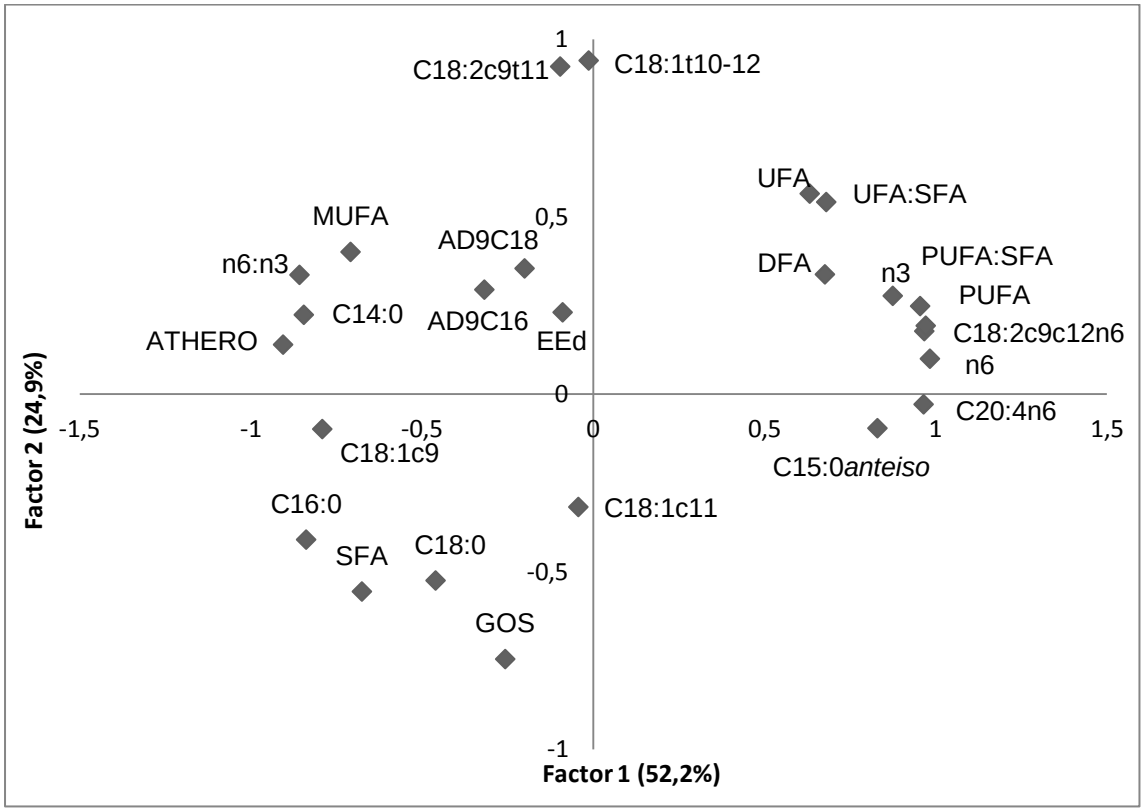

Figure 5.1 - Two principal factors of fatty acids profile of meat of Santa Ines lambs fed with cottonseed (whole, meal and high oil meal) and a control group without cottonseed during 95 days before slaughter. SFA: Saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n6: n-6 fatty acids; n3: n-3 fatty acids; n6:n3: n-6 divided by n-3; UFA:SFA: unsaturated fatty acids divided by saturated; PUFA:SFA: polyunsaturated fatty acids divided by saturated; DFA: desirable fatty acids; GOS: gossypol level in diet; EEd: ether extract in diet; AD9C16, AD9C18, ELONGASE: Δ^9-desaturase Cis-9 C16 activity, Δ^9-desaturase Cis-9 C18 activity, elongase enzyme activity, respectively, determined as described by Oliveira et al. (2011) using mathematical indices; ATHERO: atherogenicity index was calculated in accordance with the method of Ulbricht and Southgate (1991).

Table 5.8 - Regression equations between each fatty acid of meat from Santa Inês lambs and fatty acids of experimental concentrates

Fatty acid of meat		**Equation with fatty acids of concentrate**	**R^2**	***P***
C15:0 *iso*	=	0.077 + 0.073*C14:0	0.33	0.004
C16:0 *iso*	=	0.121 - 0,228*C21:0	0.22	0.024
C16:0	=	19.474 + 0.012*n-6:n-3	0.30	0.007
C17:0	=	0.756 – 1.547*C18:2t11c15	0.44	0.001
C17:1	=	0.459 – 3.398*C15:0	0.27	0.012
C18:0	=	33.196 – 0.512*MUFA	0.45	0.001
C18:1 t6-t7-t8-t9	=	0.096 + 18.481*C8:0	0.67	0.0001
C18:1 t10-t11-t12	=	-0.795 + 147.754*C8:0	0.80	0.0001
C18:1 c13	=	1.894 – 0.028*C18:2c9c12 + 8.172*C20:2	0.82	0.001
C18:1 t16	=	0.181 + 0.229*C6:0	0.58	0.0001
C18:1 c15	=	0.015 + 0.083*C21:0	0.32	0.005
C18:2 t11c15	=	0.065 + 1.013*C18:2t11c15	0.21	0.030
C20:0	=	0.162 – 2.780*C17:0 *iso*	0.30	0.007
C18:3 n3	=	0.574 – 13.682*C17:0 *iso*	0.57	0.0001
C18:2 c9t11	=	-0.294 + 44.099*C8:0 – 14.579*C22:2	0.81	0.001
C24:0	=	0.023 – 61.706*C13:0 *anteiso*	0.23	0.022
C20:5 n3	=	0.256 – 6.615*C17:0 *iso*	0.21	0.029
C22:5 n3	=	0.293 + 77.328* C13:0 *anteiso*	0.21	0.029
C22:6 n3	=	0.033 + 9.694*C13:0	0.34	0.003
SFA	=	63.237 – 0.793*C18:1c9	0.49	0.0001
UFA	=	35.272 + 0.701*C18:1c9	0.49	0.0002
n-3	=	1.569 – 36.226*C17:0 *iso*	0.30	0.007
n-6:n-3	=	8.455 + 326.445*C17:0 *iso*	0.46	0.0001
UFA:SFA	=	0.416 + 0.032*C18:1c9	0.47	0.0001
DFA	=	74.562 – 137.337*C17:0 *iso*	0.27	0.011
D9C18	=	69.759 – 799.363*C12:0	0.20	0.032

Showed only regressions with $R^2 > 0.2$. R^2: determination coefficient; *P*: p-value; SFA: Saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n6: n-6 fatty acids; n3: n-3 fatty acids; n6:n3: n-6 divided by n-3; UFA:SFA: unsaturated fatty acids divided by saturated; PUFA:SFA: polyunsaturated fatty acids divided by saturated; DFA: desirable fatty acids; , D9C18: Δ^9-desaturase Cis-9 C18 activity determined as described by Oliveira et al. (2011) using mathematical indices.

5.4. Discussion

Animals from WCS group had lower HCW, CCW and Rib eye area than CSM group and lower Y than CTL group. Therefore, this product may impact negatively on animal growth and muscle development. The data from the 12th rib showed that animals fed with CSC had lower bone mass than CTL, which may indicate a negative effect of this product on bone formation and development. In studies with humans and rats, the gossypol is reported as inducing hypokalemia due its effects on renal 11β-hydroxysteroid dehydrogenase (CHEN et al., 2009), which may explain this adverse effect in bone development. However, in present study, there is no gossypol effect in these traits. Therefore, as diets had similar composition of mineral and protein, it is not clear what led to these differences in slaughter weights and 12th rib composition. And this should be investigated further.

The composition of the 12th rib and *Longissimus dorsi* muscle showed that animals did not differ in maturity grade at slaughter between treatments. Differences in fatty acid profile of meat due to different fat content in meat between treatments were therefore not expected. This was proved in analysis of variance of meat's ether extract level, where no significant (P<0.05) effect on any fatty acid in meat was seen.

The meat from WCS group showed higher SFA than others, which was mainly related to C18:0. Despite the public concerns about overall saturated fatty acids, the stearic acid (C18:0) does not appear to affect cholesterol concentrations in human blood. Only, Myristic (C14:0) and palmitic (C16:0) acids seem to have hypercholesterolemic properties (DALEY et al., 2010). The WCS group had higher C16:0 than CTL and CSC. Therefore, comparing with these groups, WCS group may have a negative impact in fatty acid profile in terms of human health.

The C18:1c9 (oleic acid) in the diet was determinant for SFA and UFA content in meat, so the higher level of C18:1c9 led to a higher level of UFA (Table 5.8). This can be explained by the fact that monounsaturated fatty acids are preferentially absorbed over saturated fatty acids. Diets with a high level of this fatty acid mean that a high amount of these are absorbed without passing through rumen biohydrogenation. Also, oleic acid can be sterified to a triacylglycerol and incorporated in the fat droplet inside the adipocytes as energy storage (WOODS; FEARON, 2009). This also can explain partially the high SFA found in WCS group (the lowest level of C18:1c9 between diets).

Meat from all treatments had a higher PUFA:SFA ratio (0.31) compared to 0.1 level normally seen in lamb meat (WOOD et al., 2004). The literature states that the mainly reason

to variations in PUFA:SFA ratio in meat is animal fatness (DE SMET; RAES; DEMEYER, 2004). For example, the PUFA:SFA ratio in beef can be close to 0.05 in fat breeds and can rise above 0.5 in very lean breeds, such as double-muscled animals (DE SMET; RAES; DEMEYER, 2004). In the present study, the fat content in muscle was equal to 7.77 g/100 g of fresh muscle, not differing between treatments. Therefore, the animals can be considered in a medium to high fatness state, which not justified the high PUFA:SFA ratio encountered.

As shown in table 5.2, the PUFA content in all concentrates were next to 50% and the PUFA:SFA ratio was between 1.50 and 3. Therefore, a high PUFA level in diets may produce an overload of biohydrogenation system of rumen, which leads to higher absorption and deposition of PUFA in meat. These hypotheses disagree with De Smet, Raes and Demeyer (2004) which stated that this ratio is mainly influenced by genetics and, principally, by animal fatness, and much less by nutrition.

Landim et al. (2011) analyzing the fatty acid profile of meat from animals of the same breed and at the same slaughter weight as our study, found more monounsaturated fatty acids (44.12% versus 38.15% in this study) and much less polyunsaturated fatty acids (1.59% versus 13.38% in this study), what may be due to high level of n-6 fatty acids in the meat of this study and agree with the hypotheses that the diets led to high level of PUFA as discussed previously. The high level of n-6 fatty acids was also seen when comparing our results to other authors, as, for example, in our study, the means of C18:2n-6 and C20:4n-6 was 8.16 and 3.23, while Ponnampalam et al. (2002) found 3.72 and 1.19, respectively. This can be explained by the high level of C18:2c9c12 in the experimental diets, close to 50%, and the high level of fat in the diets, which led to lower ruminal biohydrogenation.

The values obtained for C18:1t10-t11-t12 (*trans* octadecenoic fatty acid) were greater in meat from the CSC group and the meat from WCS group showed the lowest value. All *trans* octadecenoic fatty acids are principally formed by vaccenic acid (*trans*-11 C18:1), an intermediary product of rumen biohydrogenation of linoleic (18:2n-6) and linolenic (18:3n-3) acids (OLIVEIRA et al., 2011). The lowest proportion of C18:1t10-t11-t12 in the WCS group may have been related to a lower availability of n-3 fatty acids in rumen due to lower levels of these in this diet (Table 5.2) as n-3 fatty acids are preferred to biohydrogenation process than the others (KIM et al., 2007). Moreover, the fatty acids in whole cottonseed have a greater protection from rumen microorganisms than other diets that had lipids directly exposed to biohydrogenation. The results from correlation, regression and factor analysis support a negative effect of gossypol concentration in diet on vaccenic acid (C18:1t11). More, the C8:0

in diets had a positive effect on vaccenic acid. Therefore, these compounds can also explain partially the differences between treatments, but further studies are needed to explain these relationships.

Biohydrogenation of vaccenic acid occurs slowly, resulting in an increased concentration in the rumen, making it available for absorption in the intestinal tract (BAUMAN et al., 2011). The increased ruminal concentration of vaccenic acid may cause an increase in CLA in fatty tissue due the action of the Δ^9-desaturase enzyme. The c9t11CLA content in the CSM and CSC groups (1.02 and 1.26 g/100 of total fatty acids, respectively) was higher than the others, and also was higher than reported by Ponnampalam et al. (2002) and Raes, De Smet and Demeyer (2004), who affirmed that c9t11CLA content in beef and lamb meat varied between 0.2 and 1.0 g/100g of total fatty acids.

These results are contrary to Raes, De Smet and Demeyer (2004), who found that the CLA content did not undergo a great increase due to nutritional factors. The increased production of its precursor t11C18:1 (vaccenic acid) in the rumen during biohydrogenation of C18:2n-6 and C18:3n-3 generally is responsible for these higher c9t11CLA levels (RAES; DE SMET; DEMEYER, 2004). Nevertheless, as CSM and CSC diets did not show higher levels of these fatty acids in relation to the CTL, other factors may have stimulated the deposition of c9t11CLA in meat from these groups. The regression analysis showed that gossypol in the diet negatively impaired the CLA content in meat. On the other hand, the CLA content in meat was positively related to C8:0 and negatively to C22:2 in the diet. Further, this may explain the differences between the CTL group, and CSM and CSC groups. More investigation is necessary to confirm these hypotheses and to clarify the physiological pathways that explain these relationships.

De novo synthesis of CLA from vaccenic acid (t11C18:1) also has been documented in humans metabolism and the rate of conversion has been estimated to range from 19 to 30%. According to Daley et al. (2010), true human dietary intake of CLA should consider native c9t11C18:2 (actual CLA) as well as the t11C18:1 (potential CLA) content of foods. Thus, meat from the CSC group showed a very high level of this true CLA (6.65%), while meat from CSM and CTL groups had an intermediate level (4.80% and 4.13%, respectively), and meat from WCS group showed a very low level (1.41%). So, looking for human health issues, the WCS group again demonstrated a worse result.

All groups showed a high mean n-6 to n-3 ratio (mean equal to 15.82) compared to other experiments with other diets (PONNAMPALAM et al., 2002; LANDIM et al., 2011). However, the results of our study are very similar to other that used whole cottonseed in the

lamb diet (MADRUGA et al., 2008). The fatty acid profile of experimental concentrates also showed a high n-6 to n-3 ratio. This demonstrated that these diets with high oil level (both soybean and cottonseed) led to high n-6 deposition rather than n-3 deposition. In relation to a healthy human diet, the n-6 to n-3 ratio recommended is 4 or less (AFMAN; MULLER, 2012), therefore all meat from this study had ratios well above this limit.

The meat from CTL group had higher n-3 fatty acid content and lower n-6 to n-3 ratio than others. This demonstrated a worse fatty acid profile in meat from animals that received cotton co-products, which agrees with results found by Madruga et al. (2008).

Oliveira et al. (2011), evaluating the effects of dietary addition of ground oilseed sources on the quality and fatty acid profile of meat from zebu steers, concluded that meat from animals fed with cottonseed had the least desirable characteristics because of the greater concentrations of SFA, in particular myristic and palmitic acids. Moreover, as found in our study, those authors observed that meat from animals fed ground cottonseed had the lowest n-3 and the highest n-6 to n-3 ratio.

The Δ^9-C18 desaturase enzyme activity index was lower in WCS group than CSM group, demonstrating that this diet may impair activity of this enzyme. The gossypol in diet had no effect in this enzyme activity. The effect of feeding system on the expression of genes related to fat metabolism was recently demonstrated (DERVISHI et al., 2010; 2011). The activity of Δ^9-C18 desaturase enzyme is affected by some factors, but insulin is considered the principal influencing hormone (PALMQUIST; MATTOS, 2006). Therefore, the activity of Δ^9-C18 desaturase enzyme may be related to a direct effect of diets on gene expression or on hormones. Nevertheless, further studies are needed to investigate these hypotheses.

Albeit the importance of fatty acids to human health, fat properties (chemical and physical) also affect sensorial qualities and preservation of meat. UFAs are particularly important in flavour development and the effect of fatty acid on meat flavour is due to the production of volatile, odorous, lipid oxidation products during cooking and the involvement of these with Maillard reaction products to form other volatiles which contribute to odour and flavour (WOOD et al., 2004). Unsaturated fatty acid levels were positively correlated to *cowy*, *cardboard*, *painty*, and *livery* flavors that are unsatisfactory for beef palatability (CAMFIELD et al., 1997). When UFA levels become too high, off-flavors can develop, especially during cooking (ELMORE et al., 2002). As already discussed, a high level of PUFA was observed in the meat of this study (for all groups), which can decrease its shelf life and affect characteristics associated with color and flavor (OLIVEIRA et al., 2011). Therefore, the type

of diet used in this study (high fat content with high soybean and/or cottonseed oil) may change the organoleptic characteristics of meat and the shelf life of this product.

The high level of C18:2n-6 also found in meat of this study may suffer rapidly oxidation when heated, producing volatile compounds, such as pentanal and hexanal aldehydes, that compromise the aromatic meat quality (WOOD et al., 2004). Hexanal and 2,4-decadienal contribute positively to beef flavor, but may produce undesirable flavors at higher concentrations. These two compounds are produced in the greatest amounts during oxidation of C18:2 during heating, as well as overshadowing compounds that also help produce typical beef flavors (CALKINS; HODGEN, 2007). Agreeing with it, Fisher et al. (2000) demonstrated that Suffolk lambs fed concentrates had a high concentration of C18:2 (linoleic acid) and its major product C20:4 (arachidonic acid) and had a high score for abnormal lamb flavour.

In this study, the meat from the CSC group had the highest level of C18:1t11 (vaccenic acid), a fatty acid implicated in off-flavors (CAMFIELD et al., 1997). Consequently, this also may compromise the meat quality from these animals.

The melting point of lipids and the firmness/hardness of carcass fat is closely related to the concentration of stearic acid (C18:0) (WOOD et al., 2004). In 1000 lambs, Wood et al. (2004) found that the concentration of C18:0 showed the highest correlation with melting point. Therefore, the meat from the WCS group (the highest C18:0 level) may have had a higher melting point. This can lead to less fat melted in the mouth of consumer, which tends to increase the sticky feeling in the mouth sometimes felt after eating lamb (WOOD et al., 2004).

Odour and flavour alteration in meat attributed to animals fed with whole cottonseed has been a topic of great discussion in the Brazilian meat industry. The majority of complaints refer to a liver-like taste. This also is a flavor problem currently facing beef producers in the US (CALKINS; HODGEN, 2007). There is speculation that the increase in incidence of liver-like samples may be attributed to changes in feeding practices. And, in the US, wet distiller's grain plus solubles (WDGS) is pointed as one possible candidate. Medium and long chain unsaturated fatty acids play a role in creating this liver-like off-flavor, in the regression models, the UFA alone accounted for 40% of the variation in liver-like off-flavor (CALKINS; HODGEN, 2007). Indeed, the fatty acid profile of liver have a PUFA:SFA close to 1 and PUFA represents close to 40% of fatty acids (KIM et al., 2007). Therefore, this fatty acid profile may contribute to its flavor.

In the present study, we showed that the whole cottonseed is not related to higher PUFA than others groups. And yet, the whole cottonseed did not have any difference in fatty acid profile that supports the attribution of liver-like off-flavor to this product specifically. Otherwise, as discussed previously, all our experimental diets had a high PUFA level compared to other studies and, consequently, produced a meat with high PUFA level. In fact, generally, the diets formulated with whole cottonseed or WDGS have high levels of PUFA than diets without these products, so it seems that the real cause of liver-like off-flavor is the high PUFA level in meat. Thus, these products are pointed as causing this change due to the widespread use of these in raw form, which led to high PUFA diets. Therefore, further understanding of manipulation of unsaturated fatty acids in muscle tissue is needed to avoid off-flavors incidence.

As shown previously, C17:0 *iso* in the diet had a negative regression with C18:3n3, C20:5n3, total n-3 and a positive regression with n-6:n-3 ratio in meat, despite the low level of this fatty acid in diets (mean equal to 0.036g/100g of fat). This may indicate that C17:0 *iso* had an impact on physiological pathways of these n-3 fatty acids. According to Palmquist and Mattos (2006) the fatty acids of odd carbon chain and *iso* can be used as indicators to estimate the microbial colonization of roughage particles. It is possible that higher C17:0 *iso* led to higher microbial colonization, promoting more biohydrogenation and then less n-3 was absorbed and deposited in muscle. Little information is available in the literature about the metabolic impact of specific fatty acids and, specially, about *iso* and *anteiso* forms of each fatty acid in the diet of ruminants. Thus, this result highlighted the importance of further studies in this topic.

5.5. Conclusions

Feeding lambs with cotton co-products produce meat with lower n-3 fatty acid content and worse n-6 to n-3 ratio, which is not interesting in terms of human health. Nevertheless, meat from animals that received cottonseed meals has the highest levels of CLA. And the meat from animals that received whole cottonseed has high saturated fatty acids and also low level of the true CLA (CLA + vaccenic acid). Therefore, between cotton co-products, the recommendation is that the meals (both low and high oil) must be preferred for use in ruminant feed rather than whole cottonseed.

References

AFMAN, L.; MULLER, M. Human nutrigenomics of gene regulation by dietary fatty acids. **Progress in Lipid Research**, Amsterdam, v. 51, n. 1, p. 63-70, 2012. ISSN 0163-7827.

AOAC. **Official methods of analysis of the AOAC**. 16th. ed. Arlington: AOAC International, 1995.

BAUMAN, D. et al. Nutrigenomics, Rumen-Derived Bioactive Fatty Acids, and the Regulation of Milk Fat Synthesis. **Annual Review of Nutrition**, Palo Alto, v. 31, p. 299-319, 2011. ISSN 0199-9885.

CALKINS, C.; HODGEN, J. A fresh look at meat flavor. **Meat Science**, Barking, v. 77, n. 1, p. 63-80, 2007. ISSN 0309-1740.

CAMFIELD, P. et al. Effects of frame size and time-on-feed on carcass characteristics, sensory attributes, and fatty acid profiles of steers. **Journal of Animal Science**, Albany, v. 75, n. 7, p. 1837-1844, 1997. ISSN 0021-8812.

CHEN, B. et al. The (+)- and (-)-gossypols potently inhibit human and rat 11 beta-hydroxysteroid dehydrogenase type 2. **Journal of Steroid Biochemistry and Molecular Biology**, Amsterdam, v. 113, n. 3-5, p. 177-181, 2009. ISSN 0960-0760.

CRAWFORD, M. et al. Comparative studies on fatty acid composition of wild and domestic meats. **International Journal of Biochemistry**, Bristol, v. 1, n. 3, p. 295-305, 1970. ISSN 0020-711X.

DALEY, C. et al. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. **Nutrition Journal**, London, v. 9, p. 10, 2010. doi:10.1186/1475-2891-9-10. ISSN 1475-2891.

DE SMET, S.; RAES, K.; DEMEYER, D. Meat fatty acid composition as affected by fatness and genetic factors: a review. **Animal Research**, Les Ulis, v. 53, n. 2, p. 81-98, 2004. ISSN 1627-3583.

DERVISHI, E. et al. Effect of the feeding system on the fatty acid composition, expression of the Delta(9)-desaturase, Peroxisome Proliferator-Activated Receptor Alpha, Gamma, and Sterol Regulatory Element Binding Protein 1 genes in the semitendinous muscle of light lambs of the Rasa Aragonesa breed. **BMC Veterinary Research**, London, v. 6, p. 40, 2010. doi:10.1186/1746-6148-6-40. ISSN 1746-6148.

_____. The effect of feeding system in the expression of genes related with fat metabolism in semitendinous muscle in sheep. **Meat Science**, Barking, v. 89, n. 1, p. 91-97, 2011. ISSN 0309-1740.

EL-SHARAKY, A. et al. Spermatotoxicity, biochemical changes and histological alteration induced by gossypol in testicular and hepatic tissues of male rats. **Food and Chemical Toxicology**, London, v. 48, n. 12, p. 3354-3361, 2010. ISSN 0278-6915.

ELMORE, J. et al. Effect of lipid composition on meat-like model systems containing cysteine, ribose, and polyunsaturated fatty acids. **Journal of Agricultural and Food Chemistry**, Easton, v. 50, n. 5, p. 1126-1132, 2002. ISSN 0021-8561.

FISHER, A. et al. Fatty acid composition and eating quality of lamb types derived from four diverse breed x production systems. **Meat Science**, Barking, v. 55, n. 2, p. 141-147, 2000. ISSN 0309-1740.

FOLCH, J.; LEES, M.; STANLEY, G. A simple method for the isolation and purification of total lipides from animal tissues. **Journal of Biological Chemistry**, Baltimore, v. 226, n. 1, p. 497-509, 1957. ISSN 0021-9258.

HARA, A.; RADIN, N. Lipid extraction of tissues with a low-toxicity solvent. **Analytical Biochemistry**, New York, v. 90, n. 1, p. 420-426, 1978. ISSN 0003-2697.

KIM, S. et al. Effects of dietary n-6 : n-3 fatty acid ratio on feed intake, digestibility, and fatty acid profiles of the ruminal contents, liver, and muscle of growing lambs. **Journal of Animal Science**, Albany, v. 85, n. 3, p. 706-716, 2007. ISSN 0021-8812.

LAI, L. et al. Generation of cloned transgenic pigs rich in omega-3 fatty acids. **Nature Biotechnology**, New York, v. 24, n. 4, p. 435-436, 2006. ISSN 1087-0156.

LANDIM, A. et al. Fatty acid profile of hair lambs and their crossbreds slaughtered at different weights. **Tropical Animal Health and Production**, Edinburg, v. 43, n. 8, p. 1561-1566, 2011. ISSN 0049-4747.

MADRUGA, M. et al. Effect of diets with increasing levels of whole cotton seed on chemical composition and fatty acid profile of Santa Inez (Santa Ines) lamb meat. **Revista Brasileira De Zootecnia**, Viçosa, v. 37, n. 8, p. 1496-1502, 2008. ISSN 1516-3598.

MERTENS, D. et al. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. **Journal of AOAC International**, Arlington, v. 85, n. 6, p. 1217-1240, 2002. ISSN 1060-3271.

MOH, P. P. et al. Effect of gossypol on 5-alpha-reductase and 3-mu-hydroxysteroid dehydrogenase-activities in adult-rat testes. **Research Communications in Chemical Pathology and Pharmacology**, New York, v. 82, n. 1, p. 12-26, 1993. ISSN 0034-5164.

NRC. National Research Council. **Nutrient requirements of small ruminants**: sheep, goats, cervids, and new world camelids. Washington, DC: The National Academies Press, 2007. ISBN 9780309102131.

OLIVEIRA, D. et al. Fatty acid profile and qualitative characteristics of meat from zebu steers fed with different oilseeds. **Journal of Animal Science**, Albany, v. 89, n. 8, p. 2546-2555, 2011. ISSN 0021-8812.

PALMQUIST, D. L.; MATTOS, W. R. S. Metabolismo de lipídeos. In: BERCHIELLI, T. T. et al. (Ed.). **Nutrição de ruminantes**. Jaboticabal: FUNEP, 2006. cap. 10, p.287-310.

PARIZA, M.; PARK, Y.; COOK, M. The biologically active isomers of conjugated linoleic acid. **Progress in Lipid Research**, Amsterdam, v. 40, n. 4, p. 283-298, 2001. ISSN 0163-7827.

PONNAMPALAM, E. et al. Effects of dietary lipid type on muscle fatty acid composition, carcass leanness, and meat toughness in lambs. **Journal of Animal Science**, Albany, v. 80, n. 3, p. 628-636, 2002. ISSN 0021-8812.

RAES, K.; DE SMET, S.; DEMEYER, D. Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. **Animal Feed Science and Technology**, Amsterdam, v. 113, n. 1-4, p. 199-221, 2004. ISSN 0377-8401.

SAEKI, K. et al. Functional expression of a Delta 12 fatty acid desaturase gene from spinach in transgenic pigs. **Proceedings of the National Academy of Sciences of the USA**, Washington, DC, v. 101, n. 17, p. 6361-6366, 2004. ISSN 0027-8424.

SIMOPOULOS, A. Essential fatty acids in health and chronic disease. **American Journal of Clinical Nutrition**, Bethesda, v. 70, n. 3, p. 560S-569S, 1999. ISSN 0002-9165.

_____. The importance of the ratio of omega-6/omega-3 essential fatty acids. **Biomedicine & Pharmacotherapy**, Paris, v. 56, n. 8, p. 365-379, 2002. ISSN 0753-3322.

ULBRICHT, T. L.; SOUTHGATE, D. A. Coronary heart disease: seven dietary factors. **Lancet**, London, v. 338, n. 8773, p. 985-992, 1991. ISSN 0140-6736.

WANG, M. Analysis of gossypol by high-performance liquid-chromatography. **Journal of Ethnopharmacology**, Lausanne, v. 20, n. 1, p. 1-11, 1987. ISSN 0378-8741.

WOOD, J. et al. Effects of fatty acids on meat quality: a review. **Meat Science**, Barking, v. 66, n. 1, p. 21-32, 2004. ISSN 0309-1740.

WOODS, V.; FEARON, A. Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: A review. **Livestock Science**, Amsterdam, v. 126, n. 1-3, p. 1-20, 2009. ISSN 1871-1413.

6. FINAL REMARKS

In this study, all cotton co-products had a negative impact on reproductive system of puberal lambs. Therefore, the reproductive problems are not related only to whole cottonseed as is widely disclosed. Thus, it is not recommended the use of these feedstuffs for nutrition of breeding young males.

As the number of segmental aplasia of mitochondrial sheath increase according to higher gossypol concentration, the gossypol reproductive damages can be controlled through frequent gossypol analysis of cotton products before use it. Moreover, in further studies, it is very important to determine the gossypol level in plasma in order to prove its absorption. Hence, it is necessary the development of fast and cheap methods for gossypol determination in feedstuffs and plasma.

As some studies have been demonstrated the reversibility of reproductive damages, feeding livestock with these products out reproductive season can be applicable. However, further studies are need in this topic.

This study was not able to identify any changes in fatty acid profile of meat from animals that received whole cottonseed that could be related to flavor alterations. Therefore, this product specifically not seems be related with any flavor alterations. As discussed in chapter 5, if it has some relationship with diets containing whole cottonseed in commercial feedlots, so it could be related to high unsaturated fatty acid content, which was not tested in our study as all our diets have high content of it.

Meat from animals that received cottonseed meals had the highest levels of CLA. And meat from animals that received whole cottonseed had high saturated fatty acids and also the lowest level of the true CLA (CLA + vaccenic acid). Therefore, the meals (both low and high oil) must be preferred for use in ruminant feed, which may valorize these co-products, reducing production cost of biodiesel and attending its crescent demand.

Nevertheless, lambs fed with cotton co-products produced meat with lower n-3 content and worse n-6 to n-3 ratio. Perhaps, it may impact the utilization of these products due to current high importance given to this fatty acid in human nutrition.

7. CONSIDERAÇÕES FINAIS

No presente estudo, os co-produtos do algodão apresentaram impacto negativo no sistema reprodutivo de cordeiros na fase da puberdade. Portanto, os problemas reprodutivos não estão relacionados somente com o caroço de algodão como é amplamente divulgado. Dessa forma, não se recomenda o uso desses alimentos para nutrição de machos jovens destinados a reprodução.

Como o número de aplasia segmentar da bainha mitocondrial foi crescente de acordo com a concentração de gossipol na dieta, os danos reprodutivos causados pelo gossipol podem ser controlados através da análise frequente do teor de gossipol nesses produtos antes de usá-los. Além disso, em pesquisas futuras, é muito importante determinar a concentração de gossipol no plasma de forma a comprovar a absorção deste. No entanto, é necessário o desenvolvimento de métodos rápidos e baratos de determinação da concentração de gossipol tanto nos alimentos quanto no plasma.

Além disso, como vários estudos tem demonstrado a reversibilidade dos danos reprodutivos, a alimentação dos animais com estes produtos fora da estação reprodutiva pode ser aplicável. No entanto, mais estudos precisam ser realizados neste tópico.

Este estudo não foi capaz de identificar nenhuma mudança no perfil de ácido graxo da carne dos animais que receberam caroço de algodão que possa estar relacionada com alterações organolépticas. Portanto, este alimento especificamente não está relacionado com nenhuma alteração deste tipo. Como discutido no capítulo 5, se esta alteração tem alguma relação com dietas contendo caroço de algodão em confinamentos comerciais, esta pode ser consequência do alto teor de ácidos graxos insaturados, o que não foi testado neste estudo, pois todas as dietas tinham alto teor destes.

A carne dos animais que receberam farelo e torta de algodão teve um elevado teor de CLA. E a carne dos animais que receberam caroço teve alto conteúdo de ácidos graxos saturados e o menor teor de CLA verdadeiro (CLA + ácido vacênico). Portanto, o farelo e a torta devem ser preferidos para uso na alimentação de ruminantes em detrimento do caroço, o que pode valorizar esses co-produtos, diminuindo o custo de produção do biodiesel e atendendo a demanda atual.

Os cordeiros alimentados com os co-produtos do algodão produziram carne com menor conteúdo de n-3 e pior proporção entre n-6 e n-3. Possivelmente, isto pode prejudicar a utilização destes produtos devido à grande importância dada atualmente a este grupo de ácidos graxos na nutrição humana.

APPENDIX

APPENDIX A – Regression equations of each fatty acid of meat from Santa Inês lambs fed with of cottonseed co-products, testing effects of gossipol level in diet (Gos), ether extract in diet (EEd) and ether extract in *Longissumus dorsi* muscle (EEm) (linear, quadratic and cubic effects)

Fatty acids		Regression	R^2	*P*
C15:0 *iso*	=	0.029 + 0.063*Gos	0.30	0.0067
C16:0 *iso*	=	0.039 - 0,000000480767*EEm^2	0.22	0.0227
C16:0	=	0.456 + 2.647*Gos^2	0.30	0.0066
C17:0	=	0.077 + 6.331*Gos^3	0.36	0.0025
C17:1	=	0.064 - 0,00000377*EEd^3	0.23	0.0195
C18:0	=	0.445 + 54.196*Gos^3	0.44	0.0006
C18:1 t6-t7-t8-t9	=	0.071 – 22.784*Gos^3 + 0,00000935*EEd^3	0.67	0.0036
C18:1 t10-t11-t12	=	0.164 – 89.442*Gos^3 + 0,00002949*EEd^3	0.83	0.0003
C18:1 c13	=	0.0665 – 1.2559*Gos + 75.7082*Gos^3 + 0,000000036*EEd^3	0.83	0.0001
C18:1 t16	=	0.042 – 0.049*Gos + 0,00000545*EEd^3	0.56	0.0267
C18:1 c15	=	0.01 + 0,00000269*EEd^3	0.31	0.0057
C20:0	=	0.033 – 0.777*Gos^2	0.22	0.0243
C18:3 n3	=	0.059 – 0.191*Gos	0.66	0.0001
C18:2 c9 t11	=	0.043 – 44.692*Gos^3 + 0.0054*EEd	0.84	0.0001
C22:0	=	0.0271 – 0.0001*EEm	0.32	0.0048
C20:5 n3	=	0.037 – 0.119*Gos	0.24	0.0188
C22:5 n3	=	0.063 – 0.138*Gos	0.21	0.0272
C22:6 n3	=	0.042 – 0.002*EEd	0.31	0.006
SFA	=	0.71 + 6.941*Gos^2	0.49	0.0002
UFA	=	0.822 – 6.156*Gos^2	0.49	0.0002
n-3	=	0.098 – 0.274*Gos	0.36	0.0023
UFA:SFA	=	0.113 – 1.391*Gos^2	0.49	0.0002
PUFA:SFA	=	0.061 – 0.146*Gos	0.23	0.0199
DFA	=	1.016 – 0.188*Gos	0.22	0.0248

Showed only regressions with $R^2 > 0.2$. R^2: determination coefficient; *P*: p-value. SFA: Saturated fatty acids; UFA: unsaturated fatty acids; n3: omega-3 fatty acids; UFA:SFA: unsaturated divided by saturated fatty acids; PUFA:SFA: polyunsaturated divided by saturated fatty acids; DFA: desirable fatty acids.