UNIVERSIDADE FEDERAL DE PERNAMBUCO – UFPE
CENTRO DE CIÊNCIAS BIOLÓGICAS – CCB
MESTRADO EM BIOQUÍMICA

EFEITO DE DIETA SUPLEMENTADA COM ÓLEO DE GIRASSOL SOBRE O PERFIL LIPÍDICO PLASMÁTICO E SEMINAL DE CAPRINOS

BIANKA SANTANA DOS SANTOS

RECIFE (PE) – BRASIL
2005

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EFEITO DE DIETA SUPLEMENTADA COM ÓLEO DE GIRASSOL SOBRE O PERFIL LIPÍDICO PLASMÁTICO E SEMINAL DE CAPRINOS

Dissertação apresentada para o cumprimento parcial das exigências para obtenção do título de Mestre em Bioquímica pela Universidade Federal de Pernambuco.

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RECIFE (PE) – BRASIL
2005

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Data: 28/02/2005.

Compelido pela natureza hostil,
Aprendeu a driblar a desnutrição –
sombra constante no semi-árido,
principalmente em períodos de
estiagens prolongadas, quando tudo
(gente, bicho, planta) desidrata, seca e
morre. Adaptou-se, enfim. O bode,
freqüentemente o único bem do
sertanejo, criou-se sozinho, sem nome,
sem documento, como aconteceu com o
próprio homem.

(José Augusto Bezerra)

A Deus, aos meus pais, Nelson e Irami, e aos meus irmãos, Brejnev e Bryelle, que mesmo estando longe, sei que não deixaram de torcer, por mim, um só instante. Amo muito todos vocês!

AGRADECIMENTOS

A ***Deus***, por tudo com que me vem presenteando, atendendo as minhas preces e me dando todas as condições para que eu possa realizar os meus desejos, dentre eles, o de fazer um Curso de Mestrado.

Aos meus pais, ***Nelson e Irami***, por terem compreendido e me apoiado na realização de mais uma etapa de minha vocação profissional.

A toda a minha família, em especial a minha avó, ***Dona***, e aos meus irmãos, ***Brejnev e Bryelle***, por todos os momentos de alegria que tanto proporcionam a minha vida.

A minha orientadora, ***Professora Dra. Vera Lúcia de Menezes Lima***, por ter me concedido a honra de ser sua orientanda, investindo sempre em meu futuro acadêmico-profissional e pela confiança e amizade, em mim, depositadas.

Aos meus amigos de mestrado, ***Joelma, Amanda, Aurelino, Clébia, Márcio, Mônica, Carlos, Eduardo, Ana Helena e Gilvanely***, pelo que cada um contribuiu com o meu amadurecimento pessoal e por terem suprido, em certos momentos, as saudades de casa.

A minha equipe de trabalho, meus grandes amigos, ***Anna Lenira, Dewson*** e, em especial, ***Adenor e Ricardo***, por tudo que fizeram em prol da conclusão deste trabalho.

Aos amigos do Laboratório de Química e Metabolismo de Lipídios, que lá estavam no tempo em que comecei a ser integrante desta família, ***Amanda, Ricardo, Michelle, Ana Catarina, César, Bruno, Tiago e Vítor***, por terem me acolhido de braços abertos e por todos os momentos que passamos juntos.

Aos meus amigos, ***Soraya, Marlos, Áurea, Gustavo, Edileine, Eleandro, Eduardo, Marcelo, Washington, Joel e Conceição***, por todo o companheirismo e por todas as palavras de incentivo e carinho.

A todos os ***Professores do Departamento de Bioquímica***, que tanto contribuíram com o meu crescimento profissional e com a ampliação dos meus conhecimentos.

À ***Professora Dra. Vera Cristina e ao Professor Dr. Nicácio Henrique***, pelas informações fornecidas a respeito do tema da dissertação e por ter cedido gentilmente o seu Laboratório de Produtos Naturais para a realização de alguns experimentos durante a época de reforma do Laboratório de Lipídios, respectivamente.

Aos funcionários do Departamento de Bioquímica, ***Djalma, Neide, Iolanda, Ademar, Helena, Albérico, Maria*** e, em especial, a ***Miron***, por todas as palavras de encorajamento que me fizeram partir em busca do, até então, "sonho" Mestrado, a ***Flávio e a João***, por todo o auxílio prestado na elaboração desta pesquisa e por toda a amizade concedida a minha pessoa.

Ao ***Conselho Nacional de Pesquisas e Desenvolvimento Tecnológico – CNPq***, por todo o suporte financeiro destinado à concretização deste trabalho.

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RESUMO

Na região semi-árida do Nordeste do Brasil, a produção pecuária de caprinos é uma atividade econômica importante, principalmente devido à resistência e à capacidade de adaptação que esses animais têm perante as condições climáticas dessa região. Este trabalho visa analisar o efeito de dietas simples, como o uso do óleo de girassol, sobre o perfil lipídico plasmático e seminal de caprinos, bem como verificar sua influência sobre a fertilidade desses pequenos ruminantes. Para tanto, vinte e sete caprinos machos adultos da raça Anglo-Nubiana, com 2 a 4 anos de idade e peso médio de 54,04 ± 8,67 Kg, foram selecionados e mantidos em instalações individuais, à temperatura ambiente. Esses animais foram divididos em 3 grupos, de acordo com o percentual do conteúdo lipídico administrado (2 %, 4 % e 6 % de óleo de girassol). Os lipídios circulantes e seminais foram determinados antes e após 30 e 60 dias de administração das dietas. Também foram realizados o teste hipoosmótico e a contagem do número de espermatozóides. Os níveis plasmáticos de colesterol total foram elevados em todos os animais após a suplementação lipídica, entretanto, no sêmen, estes níveis não sofreram alterações nos caprinos sob dieta com 4 % de óleo de girassol. De modo geral, os níveis de triglicerídios não sofreram alterações, mas os de fosfolipídios totais aumentaram. Diminuição dos valores de lisofosfatidilcolina e aumento significativo dos valores de fosfatidilcolina foram observados nos caprinos sob dieta de 2 % de óleo de girassol, contudo a esfingomielina e glicoesfingolipídios só aumentaram naqueles suplementados com 4 %. Através do teste hipoosmótico, foi observado aumento significativo na percentagem dos espermatozóides reativos ao meio, em todos os grupos. Os resultados sugerem haver uma influência positiva da suplementação com óleo de girassol sobre a fluidez da membrana espermática, justificada pelo aumento dos principais fosfolipídios de membrana celular. As alterações lipídicas também podem ter tido um papel significativo no processo de espermatogênese, pois a concentração de espermatozóides no ejaculado foi aumentada significativamente. Então, os resultados do presente trabalho sugerem que suplementando a dieta de caprinos machos com 2 % de óleo de girassol é suficiente para melhorar seu desempenho reprodutivo.

ABSTRACT

In the semi-arid region of northeast Brazil, the production of caprine is a major economical activity, especially due to the resistance and adaptation ability shown by these animals under semi-arid climate conditions. This work aims to investigate the effect of sunflower oil supplementation on plasma and semen lipid profiles, in order to evaluate whether it is important for the fertility of these small ruminants. Twenty-seven adult Anglo-Nubian male caprines, 2 to 4 years old and mean weight 54,04 ± 8,67 Kg, were selected and kept in individual stalls, under environment temperature. These animals were split in 3 groups, according to the percentage of lipid content administered (2 %, 4 % and 6 % sunflower oil). Semen and circulating lipids were determined before and after 30 and 60 days on diet-supplement. Hypoosmotic test for spermatozoa and sperm cell count were also performed. Total cholesterol plasma levels were elevated in all animals after the lipid supplementation, but the seminal levels of cholesterol did not show modification in the goats fed 4 % sunflower oil. In average, triglycerides levels were unaltered, but total phospholipids increased. There was a decrease in lysophosphatidylcholine and a significant rise in phosphatidylcholine in the goats receiving 2 % of sunflower oil in the diet, but sphingomyelin and glycosphingolipids increased only in those animals supplemented with 4 %. The hypoosmotic test showed a significant increase in the percentage of spermatozoa reactive to the medium, in all groups. The results suggest a positive influence of sunflower oil on the sperm cell membrane fluidity, what can be explained by the increase on the main phospholipids that are components of cell membrane. These lipid changes seem to have a significant role in the spermatogenesis, since the sperm cell concentration in the ejaculate was also significantly increased. Thus, the results of present work suggest that the supplementation of the diet with 2 % sunflower oil is enough to improve the reproductive performance of male caprine.

INTRODUÇÃO

Os caprinos foram um dos primeiros animais domesticados pelo homem, provavelmente na África. Foram introduzidos no Brasil pelos colonizadores portugueses e, inicialmente, eram criados na faixa litorânea, antes de se espalharem pelo sertão da região Nordeste. Porém, como não havia muita procura pelos seus produtos, como carne, leite e couro, pois se preferia apenas os de origem bovina, esses animais acabaram diminuindo gradativamente em porte, massa e capacidade produtiva, em função das condições adversas do sertão. Em contrapartida, adquiriram alto nível de rusticidade e resistência às doenças, à escassez hídrica e alimentar (BEZERRA, 2005).

Uma das raças mais prevalentes no sertão nordestino é a Anglo-Nubiana, resultado do cruzamento de cabras Nubianas, famosas por seu potencial leiteiro, com bodes da Inglaterra. Estes caprinos chegaram ao Brasil desde a década de 1930, e são praticamente os antepassados da maioria do rebanho brasileiro considerado como sem raça definida – SRD (AGROPECUÁRIA TROPICAL, 2003a) (**Figura1**).

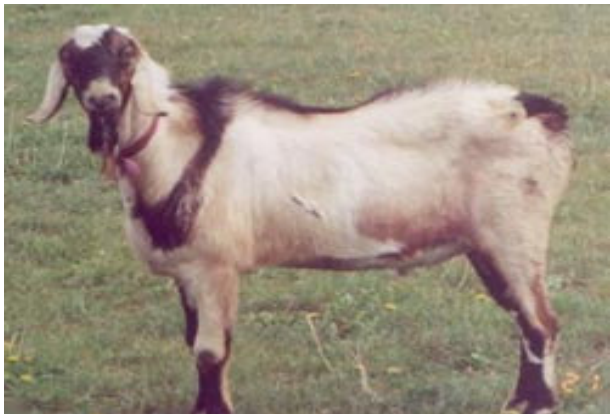

Figura 1. Caprino macho da raça Anglo-Nubiana.
Fonte: http//:www.edvethd.com.br/imagesAN%20macho, 2005**.**

Os Anglo-Nubianos se adaptam bem ao regime de estabulações ou semi-estabulações, possuem orelhas longas e caídas, pescoço e corpos alongados, pernas finas e compridas, pêlos curtos e sedosos. Porém, a quantidade de Anglo-Nubianos criados em

sistema aberto, expostos às vicissitudes do ambiente sertanejo, é alta, o que faz com que suas características raciais sejam modificadas, diminuindo seus potenciais econômicos, como por exemplo, a diminuição de seu aproveitamento leiteiro e como fonte alimentar (AGROPECUÁRIA TROPICAL, 2003b).

Então, devido à precariedade de tecnologia aliada à não utilização de padrões de qualidade dos produtos caprinos, entre outros fatores, ainda se verifica uma produção incipiente, principalmente quando se compara o efetivo mundial de caprinos, cerca de 600 milhões de cabeças, com o efetivo brasileiro, cerca de 11 milhões. Destes, aproximadamente, 90 % encontram-se na região Nordeste, o que demonstra que a caprinocultura não apenas pode, mas também deve, passar a desempenhar um papel sócio-econômico muito maior nesta região, como uma alternativa viável de geração de emprego e renda (FAO, 1995; CORDEIRO, 1998; FNP-ANUALPEC, 1998; SILVA, 1998; EMEPA, 1999).

Diante desses problemas e da importância da caprinocultura para o Brasil e, particularmente, para o Nordeste, atualmente tem havido um incentivo, de ações conjuntas de governos estaduais, instituições de pesquisa e criadores, na tentativa de se implementar novos conceitos de organização e gestão da unidade produtiva, bem como, a adoção de tecnologias necessárias para a inserção do caprinocultor na economia de mercado e para a promoção da qualidade de vida do homem no campo, em patamares condizentes com as exigências das organizações internacionais de desenvolvimento econômico e social (BRAGA *et al*, 2003; SIMPLÍCIO, 2005).

Assim se tem observado um maior interesse na busca de conhecimentos sobre os fatores correlacionados com a sua produção, como os fatores genéticos, ambientais e nutricionais. Destes, o aspecto nutricional é o que pode ser modificado de forma acessível aos pequenos e médios caprinocultores e, por isso, suplementações alimentares vêm sendo empregadas, na tentativa de se aumentar o suporte energético desses animais, a fim de se obter rebanhos com maior vigor físico, melhor qualidade de carcaça, carne, leite e melhor

desempenho reprodutivo (CONTRERAS *et al*, 2000; RENAVILLE *et al*, 2002; BRAGA *et al*, 2003).

Estudos relatam que os nutrientes promovem a expressão de vias metabólicas que capacitam os animais a atingirem seu potencial genético para a reprodução. Estas vias são complexas e, em muitos casos, ainda não foram elucidadas. Também se tem demonstrado o impacto da restrição e da repleção de nutrientes sobre o eixo hipotalâmico-hipofisário e, inclusive, alguns estudos formulam a hipótese de que todos os processos reprodutivos, da gametogênese à lactação, são determinados pela disponibilidade de nutrientes, ou ainda, que o desempenho reprodutivo é afetado pela mobilização, no corpo, de reservas energéticas (MARZOUKI & CONIGLIO, 1982; COSGROVE & FOXCROFT, 1996; ROBINSON, 1996; BOUKHLIQ & MARTIN, 1997; FRIGGENS, 2003; COLAZO *et al*, 2004).

Uma das fontes nutritivas mais cogitadas para aumentar o suporte energético desses animais é a lipídica, especialmente provinda de óleos vegetais, os quais são ricos em ácidos graxos essenciais. Estes ácidos não são sintetizados por caprinos e, portanto, precisam ser supridos pela dieta. Um exemplo de óleo vegetal rico em ácidos graxos essenciais é o óleo de girassol, o qual contém 68,5 % de ácido graxo poliinsaturado, conhecido como ácido linoléico (C18:2ω6). Deficiência de ácido linoléico tem sido relacionada com redução nas funções reprodutivas de algumas espécies animais. Porém, há relatos de aumento da proporção ω6:ω3 em amostras astenozoospérmicas e oligozoospérmicas de sêmen humano (VAN SOEST, 1994; SIMOPOULOS, 1996; CHARDIGNY *et al*, 1998; ZALATA *et al*, 1998; BEAM *et al*, 2000; SEMMA, 2002; BAUMAN *et al*, 2003; YEOM *et al*, 2004).

Assim o uso de lipídios na dieta e seus efeitos têm merecido muita atenção, face ao crescente interesse na utilização de suplementos lipídicos como fonte energética para rações de animais de exploração econômica. Os efeitos de suplementação lipídica sobre o perfil bioquímico de animais precisam ser melhor investigados, além de que estudos relacionando suplementação alimentar com caprinos machos, principalmente, reprodutores,

são ainda muito escassos (NORDOY, 1987; JENKINS, 1993; SEIQUER *et al*, 1995; CONTRERAS *et al*, 2000; BAUMAN *et al*, 2003; MAMAT *et al*, 2005).

Alguns estudos têm demonstrado que ácidos graxos poliinsaturados provindos da dieta são incorporados nos lipídios dos tecidos animais, diretamente ou através de processos metabólicos como dessaturação e elongação. É sabido que dietas com ácidos graxos, mais especificamente com poliinsaturados, influenciam o perfil destas moléculas nos tecidos ricos em fosfolipídios, como o cérebro e a retina. Os espermatozóides também são ricos em fosfolipídios e podem ser sensíveis à disponibilidade de ácidos graxos poliinsaturados na alimentação, porém a possível influência da composição destes tipos de ácidos da dieta sobre o conteúdo de ácidos graxos poliinsaturados do espermatozóide permanece pouco explorada (BLESBOIS *et al*, 1997; BAZINET *et al*, 2004).

Em ruminantes, há ainda uma grande discussão em torno da influência dos ácidos graxos sobre o conteúdo destas moléculas nas células e nos tecidos. Um estudo feito com o intuito de verificar a influência de óleo de soja sobre a composição de eritrócitos, em caprinos, indicou que esse nutriente não modificou a composição das células eritrocitárias após um período de 21 dias de experimento, o que foi atribuído ao metabolismo dos ácidos graxos no rúmen (uma das partes do estômago desses animais) e/ou ao pouco tempo de averiguação (JENKINS, 1993; BAUMAN *et al*, 2003; YEOM *et al*, 2004) (**Figura 2**).

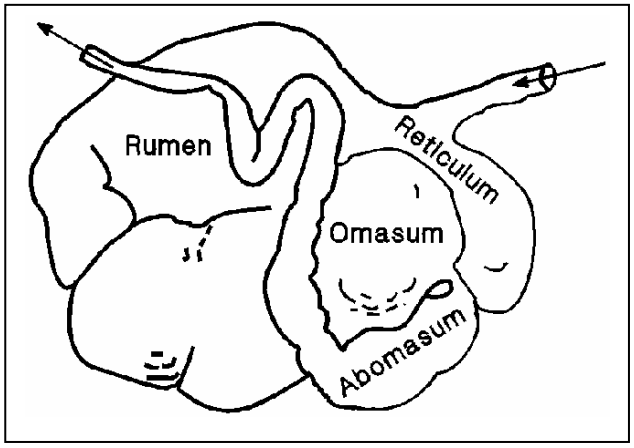

Figura 2. Estômago de ruminantes, mostrando suas 4 partes: reticulum, rumen, omasum e abomasum.
Fonte: http://www.extension. umn.edu/distribution/livestocksystems/images/0469f03.gif-rum. 2005.

O metabolismo dos lipídios provindos da dieta, no rúmen, consiste em dois processos importantes, lipólise e biohidrogenação, e ocorre devido à ação dos microorganismos presentes nesta região do estômago desses animais. A lipólise consiste na hidrólise de ligações ésteres encontradas nos triglicerídios, fosfolipídios e glicolipídios. Os triglicerídios são hidrolisados principalmente devido à ação de enzimas, lipases, produzidas por *Anaerovibrio lipolytica*, enquanto a hidrólise dos dois últimos é catalisada principalmente por lipases liberadas por *Butyrivibrio fibrisolvens*. A ação enzimática promove a liberação de ácidos graxos, os quais, quando insaturados, são biohidrogenados, tendo o número de suas duplas ligações reduzido (JENKINS, 1993; HARFOOT & HAZLEWOOD, 1997; BAUMAN *et al*, 2003) (**Figura 3**).

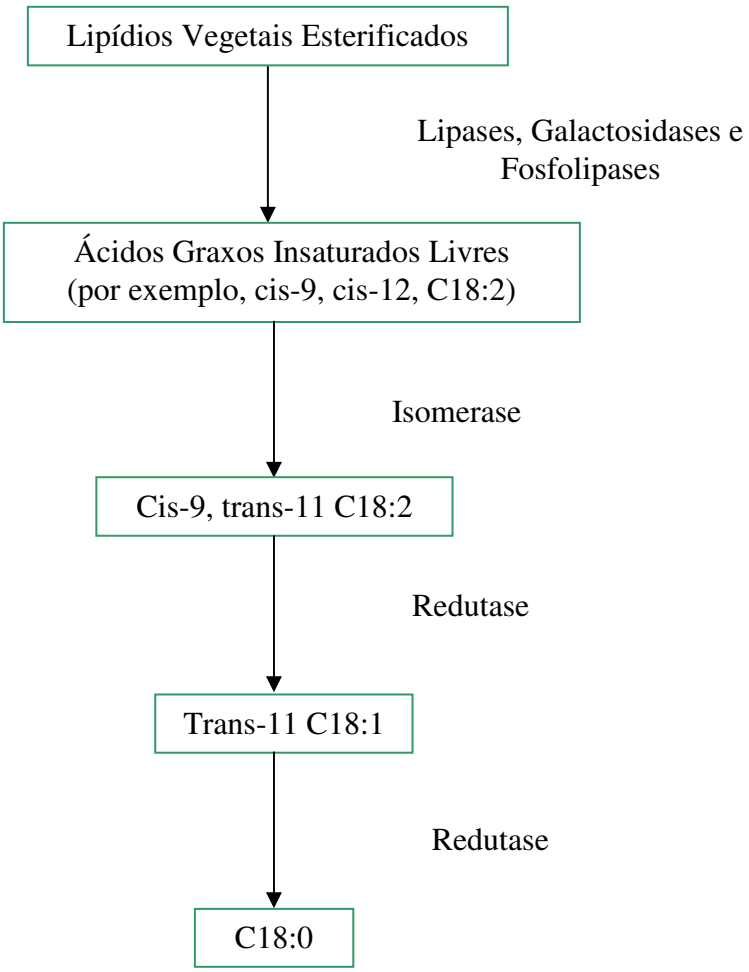

Figura 3. Passos-chave na conversão de lipídios vegetais esterificados a ácidos graxos saturados por lipólise e biohidrogenação no rúmen. Lipases, Galactosidases, Fosfolipases, Isomerase e Redutases são enzimas de microorganismos ruminais.
Fonte: Adaptado de JENKINS, 1993.

Possivelmente, este mecanismo serve para proteger os microorganismos ruminais dos efeitos tóxicos dos ácidos graxos insaturados. Mudanças na concentração de ácidos graxos insaturados devem ser realizadas com precaução, porque esta fração lipídica provavelmente determina efeitos negativos na capacidade fermentativa dos microorganismos do que outras frações, como triglicerídios e ácidos graxos saturados (SFAs). Também foi observado que a lipólise é reduzida quando o nível de gordura alimentar é elevado ou quando fatores, como a diminuição do pH ruminal, inibem a atividade e o crescimento bacterianos. Atualmente, a recomendação geral sobre a suplementação lipídica na dieta é que a quantidade total de gordura não exceda 6% a 7% da matéria seca (JENKINS, 1993; DOREAU *et al*, 1997; DEMEYER & DOREAU, 1999; NRC, 2001; BAUMAN *et al*, 2003).

No entanto, estudos sobre o efeito do aumento da concentração de ácido linoléico no rúmen são contraditórios. Alguns autores indicam que a capacidade de hidrogenação se eleva com o aumento da concentração de ácido linoléico, enquanto outros demonstram que quando há aumento deste ácido graxo, a biohidrogenação não ocorre de maneira completa e, portanto, ácidos graxos provindos da dieta podem escapar deste processo. Estudos mostram também que a adição de lipídios na dieta causa apenas uma elevação transiente nos ácidos graxos poliinsaturados abomassais e que a melhora do fluxo de ácidos graxos insaturados para o duodeno é muito limitada (HARFOOT *et al*, 1973; KELLENS *et al*, 1986; JENKINS, 1993; BEAM *et al*, 2000; LOOR *et al*, 2004).

Portanto, os efeitos específicos ocasionados pelos diferentes tipos e quantidades de ácidos graxos na dieta são complexos e difíceis de avaliar, o que demonstra que a prática de suplementação lipídica não pode ser feita sem um controle e investigação prévia, haja vista que o desejável é que lipídios sejam suplementados às dietas sem causar danos ao organismo, bem como, possam aumentar o suporte energético dos animais e melhorar, também, o seu desempenho reprodutivo (ESCUDERO *et al*, 1998; BAUMAN *et al*, 2003).

Em ruminantes que já atingiram a maturidade sexual, tem-se verificado que as respostas à nutrição podem ser divididas em dois tipos, aquelas que podem ser obtidas a

curto prazo, como a atividade testicular dependente do controle do sistema endócrino, e as que são conseguidas a longo prazo, como o crescimento testicular e a produção de espermatozóides. Há relatos de que existe um prazo de cerca de 6 a 7 semanas para se observar resposta à nutrição no número de espermatozóides, refletindo o tempo que a espermátide esférica, nas células germinais, leva para se desenvolver em espermatozóide maduro na cauda distal do epidídimo (MARZOUKI & CONIGLIO, 1982; COSGROVE & FOXCROFT, 1996).

Como as membranas plasmáticas das células somáticas, as dos espermatozóides são uma mistura heterogênea de fosfolipídios, glicolipídios e colesterol, os quais estão distribuídos assimetricamente formando as bicamadas, porém os espermatozóides são células altamente diferenciadas, polarizadas, compartimentalizadas e com uma alta proporção membrana:citoplasma, com suas membranas possuindo muitas características físicas e funcionais, organizadas em várias regiões específicas, que as tornam distintas das membranas de outras células. Espermatozóides de mamíferos apresentam domínios e diferentes ultra-estruturas em suas membranas, com divisões claras entre elas. Estes domínios têm diferentes funções e cada um contribui separadamente para o *status* do espermatozóide (APEL-PAZ *et al*, 2003) (**Figura 4**)

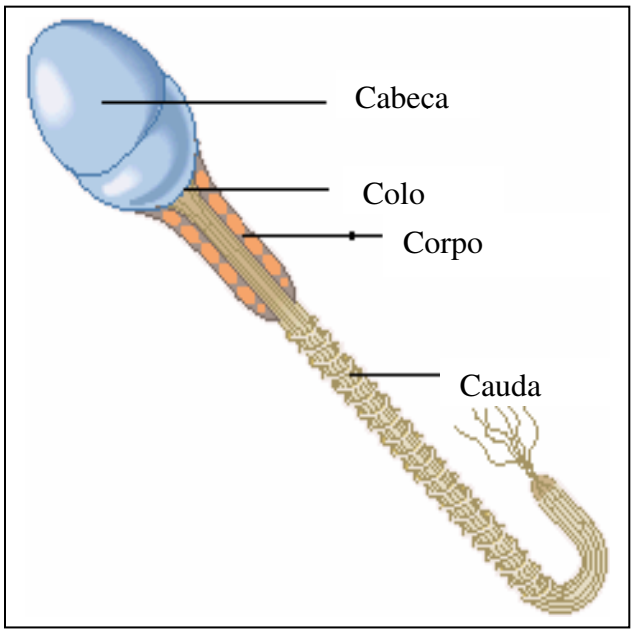

Figura 4. Espermatozóide de mamíferos e suas estruturas: cabeça, colo, corpo e cauda.
Fonte: http//:personal.telefonica.terra.es/web/flromeras/espermat_archivos/image002.gif-sperm, 2005.

Nos espermatozóides, colesterol e fosfolipídios são constituintes essenciais. A composição desses lipídios na membrana plasmática tem um efeito significativo nas propriedades físico-químicas e, por sua vez, nas características funcionais dos espermatozóides. Eles são fatores primordiais na determinação da temperatura de transição de fase e da fluidez da membrana, além de terem papéis importantes em eventos como motilidade, viabilidade, maturidade, capacitação espermática, reação acrossomal e propriedades fusiogênicas do espermatozóide com o ovócito II, bem como são importantes para o processo de congelamento-descongelamento, essencial à inseminação artificial e à pesquisa (ZALATA *et al*, 1998; GRIZARD *et al*, 2000; APEL-PAZ *et al*, 2003).

Os fosfolipídios contêm um grupo polar (cabeça) e um grupo apolar (cauda). A cauda é constituída geralmente de ácidos graxos que apresentam uma ou mais dupla ligação, isto é, insaturados. Essa dupla ligação é responsável por um dobramento na cauda fosfolipídica. A diferença no tamanho e na saturação das caudas de ácidos graxos é importante devido ao fato de influenciarem a capacidade das moléculas de fosfolipídios de se acomodarem umas nas outras, e, por esta razão, de afetarem a fluidez da membrana (FLESCH & GADELLA, 2000) (**Figura 5**).

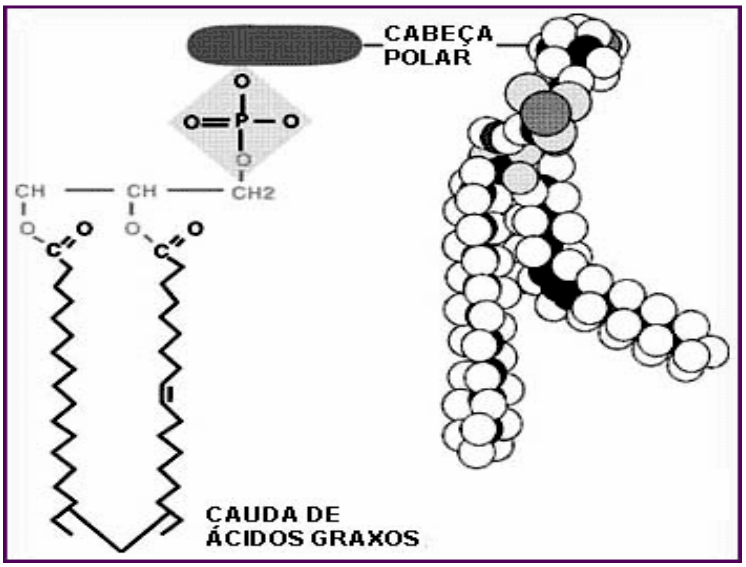

Figura 5. Esquema de fosfolipídio, evidenciando sua cabeça polar e sua cauda de ácidos graxos.
Fonte: Adaptado de SABARENSE, 2003.

A classe de fosfolipídio mais predominante nos espermatozóides é fosfatidilcolina, enquanto que no plasma seminal é a esfingomielina. Tem sido encontrado que a fluidez da membrana depende em grande parte da composição de ácidos graxos desses fosfolipídios, o que leva a crer que esses ácidos tenham um papel importante na qualidade dos espermatozóides e nos processos de fertilização. Os fosfolipídios dos espermatozóides de mamíferos possuem grandes quantidades de ácidos graxos poliinsaturados, principalmente os ácidos docosahexaenóico (C22:6ω3), docosapentaenóico (C22:5ω3) e araquidônico (C20:4ω6), havendo também ácidos graxos monoinsaturados, dentre eles os ácidos oléico (C18:1ω9) e palmitoléico (C16:1ω7), e ácidos graxos saturados, como o esteárico (C18:0) e o palmítico (C16:0). Os ácidos graxos poliinsaturados constituem aproximadamente 40% dos componentes da membrana do espermatozóide, especialmente fazendo parte da composição de fosfatidilcolina (RANA *et al*, 1991; RANA *et al*, 1993; ZALATA *et al*, 1998; GRIZARD *et al*, 2000; HENNEBERRY *et al*, 2002; APEL-PAZ *et al*, 2003; SCHILLER *et al*, 2003).

As classes dos fosfolipídios também estão relacionadas com o sexo e com o estágio reprodutivo. A membrana celular é uma interface para a comunicação entre os meios intra e extracelular. Cada espécie de fosfolipídio tem um papel diferente na regulação da atividade da membrana, então alterações das classes fosfolipídicas também podem refletir mudanças no desempenho do espermatozóide (ALBESSARD *et al*, 2001).

Acúmulo de fosfatidilcolina e fosfatidiletanolamina tem sido encontrado no ovário de alguns animais indicando maior atividade metabólica dos lipídios durante a maturação gonadal. Também tem sido observada uma correlação entre o aumento dos lipídios totais e de fosfatidilcolina. Há uma hipótese de que esta classe fosfolipídica, no sêmen, sirva como uma fonte de nutrientes essenciais para os espermatozóides, tais como, fósforo orgânico, colina e ácidos graxos, principalmente, poliinsaturados, além de participar da regulação da transmissão do potencial de ação da membrana. Deficiência de fosfatidilcolina já foi correlacionada com diminuição da fluidez da membrana (ALBERTS, 1986; MOURENTE *et al*, 1994; KATAN, 1995; MAYZAUD, 1997).

Fosfatidilcolina também pode ser convertida em glicerofosforilcolina, de duas maneiras: pela adição de fosfolipídios ao esperma, durante o trânsito epididimal, com degradação destes fosfolipídios pelos espermatozóides para formar a glicerofosforilcolina; e pela degradação dos fosfolipídios pelas células epididimais com excreção da glicerofosforilcolina no lúmen do ducto deferente. A função de glicerofosforilcolina no sêmen ainda não foi elucidada, mas se sabe que mesmo quando seus precursores, fosfatidilcolina e o plasmalógeno colina, diminuem durante o trânsito epididimal, a concentração de glicerofosforilcolina se acumula durante o trajeto, com o tecido epididimal precisando participar na formação desta classe. Foi verificado, em carneiros, que todo o conteúdo de glicerofosforilcolina é formado no epidídimo e que fosfatidilcolina das lipoproteínas sangüíneas é o precursor isotópico direto de glicerofosforilcolina no plasma seminal (HAMMERSTEDT & ROWAN, 1982).

Convém mencionar, também, que a composição lipídica dos espermatozóides não é estática e sim dinâmica, mudando durante o trajeto dos espermatozóides. Um exemplo disso é a modulação do conteúdo de colesterol, que está presente em maiores concentrações no epidídimo, onde a estabilidade e a resistência da membrana são mais necessárias, e é continuamente perdido durante o trajeto destas células ao trato reprodutivo feminino, em preparação para os eventos da fusão com o ovócito II. A distribuição dos ácidos graxos nas frações fosfolipídicas das bicamadas também passam por profundas alterações durante a maturação epididimal, verificando-se uma assimetria lipídica, a fim de que os gametas atinjam sua maturidade, o que talvez seja facilitada pela incorporação de substâncias provindas da dieta (MARZOUKI & CONIGLIO, 1982; RANA *et al*, 1991; RANA *et al*, 1993; GRIZARD *et al*, 2000; APEL-PAZ *et al*, 2003).

Também se tem relatado que alguns componentes do fluido seminal são importantes para a aquisição de fertilidade pelo espermatozóide. Esfingomielina é o fosfolipídio predominante no plasma seminal e, além disso, é um dos principais componentes, juntamente com fosfatidilcolina, da membrana externa de espermatozóides de caprinos. No sêmen, a composição lipídica da membrana espermática é dependente de uma troca com os lipídios do plasma seminal (RANA *et al*, 1993).

Desempenho reprodutivo dos espermatozóides também já foi associado ao conteúdo de glicoesfingolipídios, pois provavelmente eles servem como mediadores da reação acrossômica, estabilizando a membrana espermática, inicialmente, e migrando para a região equatorial, durante o processo de capacitação. Este processo consiste nas várias mudanças fisiológicas que levam os espermatozóides a estarem competentes para a fertilização (FLESCH & GADELLA, 2000).

Tem sido reportado que aumento na fluidez da membrana do espermatozóide está associado com a sua capacidade de fertilização. O papel da membrana espermática com o meio externo é essencial e envolve transporte de íons através da membrana, a ligação de diferentes fatores a receptores específicos e a manutenção do potencial de membrana (SINHA *et al*, 1994; PÉREZ-LLANO *et al*, 2001).

Um dos testes mais correntemente empregado para avaliar o *status* da membrana do espermatozóide é o teste hipoosmótico. Numa solução hipoosmótica, o fluido é transportado para o interior do espermatozóide, atravessando a membrana plasmática. Enquanto se tenta atingir o equilíbrio entre os meios intra e extracelular, uma membrana funcionalmente intacta se expande ao longo das fibras da cauda das células espermáticas, causando uma turgescência nesta célula. Com a expansão da membrana, a cauda fica enrolada e sofre um dobramento. Espermatozóides com defeitos na membrana não ficam túrgidos e conseqüentemente suas caudas não dobram (KUMI-DIAKA, 1993; NIE & WENZEL, 2001; PÉREZ-LLANO *et al*, 2001).

O teste hipoosmótico tem sido correlacionado positivamente com a fertilização *in vitro* e *in vivo*. Estudos anteriores demonstraram que aumento do percentual de espermatozóides de hamsters positivos ao teste hipoosmótico está correlacionado diretamente ao aumento do percentual de penetração no ovócito II. Quanto maior o percentual de espermatozóides identificado com esta característica, melhor é a qualidade da amostra de sêmen, haja vista que a integridade funcional da membrana plasmática é crítica para o metabolismo e para a função normal do espermatozóide (NIE & WENZEL 2001).

Contudo, estudos relacionando os lipídios da dieta com o *status* da membrana dos espermatozóides são escassos, apesar de serem necessários para se averiguar os efeitos de suplementações alimentares, não apenas sobre o perfil bioquímico dos animais, mas também sobre o seu desempenho reprodutivo.

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3. JUSTIFICATIVA

Em virtude da importância sócio-econômica da caprinocultura para o Brasil e, particularmente, para a região Nordeste, tem-se observado a necessidade de modificações no sistema tradicional de criação desses animais, a fim de tornar essa atividade mais rentável, aumentando sua produtividade e a potencialidade desses pequenos ruminantes.

Desse modo, desafios de diversas ordens precisam ser superados e rebanhos com maior vigor físico precisam ser obtidos. Uma das tentativas de resolução, que vem sendo difundida entre os criadores, é a suplementação da alimentação desses animais com fontes nutritivas como, por exemplo, lipídicas. Paralelamente, houve um aumento na fabricação, pela indústria alimentícia, de óleos vegetais ricos em ácidos graxos essenciais, como o óleo de girassol, devido a uma maior eficácia na sua extração, o que provocou uma elevação de sua utilização na alimentação desses animais, já que não são capazes de sintetizar esses ácidos.

Contudo, geralmente se recomenda que os níveis de gordura não excedam 6% a 7% da matéria seca da dieta de ruminantes, além de que o consumo de lipídios e seus efeitos sobre a saúde ainda são questionados, inclusive, seus efeitos sobre a capacidade reprodutiva, já que trabalhos relatam, por exemplo, que deficiência de ácido linoléico, principal componente do óleo de girassol, está relacionada com alterações nas funções reprodutivas, enquanto outros relatam o aumento de seus níveis em amostras seminais astenozoospérmicas e oligozoospérmicas de humanos.

Assim, fica demonstrada a relevância da investigação dos efeitos de dietas como o uso de óleo de girassol sobre o perfil lipídico plasmático e seminal, bem como sobre a integridade funcional da membrana dos espermatozóides, de caprinos da região Nordeste do Brasil, uma vez que esses pequenos ruminantes são muito importantes para a economia deste lugar e que estudos relacionando dietas lipídicas com o *status* da membrana espermática são praticamente inexistentes.

4. OBJETIVOS

GERAL

- Verificar o efeito de suplementação com óleo de girassol sobre o perfil lipídico plasmático e seminal e sobre a integridade funcional da membrana de espermatozóides de caprinos (*Capra hircus*).

ESPECÍFICOS

- Analisar o efeito de dietas suplementadas com 2 %, 4 % e 6 % de óleo de girassol, sobre os níveis de lipídios no plasma e no sêmen de bodes, durante um período de 60 dias;

- Verificar o efeito de suplementações com óleo de girassol sobre a fertilidade de caprinos machos;

- Observar o efeito de suplementação com óleo de girassol sobre o processo de espermatogênese de caprinos machos;

- Analisar uma possível relação entre fertilidade de caprinos machos com classes lipídicas.

5. ARTIGO CIENTÍFICO

O presente trabalho será submetido à publicação na revista **Theriogenology**, cujo fator de impacto é 1.8.

Altered plasma and semen lipids due to diet supplementation with sunflower oil is associated with sperm fertility from male goats (*Capra hircus*)

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Running title: Sunflower oil supplementation and fertility in goats

Abstract

Studies associating dietary fats with membrane spermatozoa status are scanty. This study aimed to evaluate sunflower oil effect, C18:2ω6 rich, on plasma, seminal lipid profile, and on the functional integrity of sperm membrane, in goats. Therefore, 27 Anglo-Nubian crossbred mature male goats were selected and split in 3 groups of 9 animals, that received fat supplemented diets (2 %, 4 % and 6 % sunflower oil). Lipids from plasma and semen, as well as sperm concentration were determined. Status of spermatozoa membrane was accessed by hypoosmotic swelling test. It was showed a rise in plasma total cholesterol (TC) concentration; nevertheless, in semen, TC did not increase in goats fed 4 %, demonstrating that the content of TC cannot be predicted in tissues of ruminant upon fat supplemented diet. There was an increase of phospholipid (PL) level, however a significant increase of phosphatidylcholine (PC) in goats fed 2 % fat, whilst sphingomyelin (SPH) and glycosphingolipids (GSL) increased in those fed 4 %. The percentage of reactive spermatozoa to hypoosmotic solution and sperm concentration also increased significantly. These results suggest that the addition of sunflower oil was able to influence the fluidity of spermatozoa membrane, which may be due to a better quality of its phospholipid component, especially PC, SPH and GSL, having a good source of unsaturated fatty acids. Furthermore, the diet seems to have a significant role in the spermatogenic process. Thus, 2% sunflower oil supplementation already was enough to promote an enhance in the fertility of male goats.

Key-words: sunflower oil supplementation, linoleic acid, spermatozoa membrane status, lipid classes.

1. Introduction

A greater yield in the extraction of vegetable oils, which are rich in essential fatty acids, led to an increase in their production by the food industry and caused an elevation of their use in humans and animals, especially the ones under economic exploration, such as ruminants, since they are not able of synthesizing these oils [1,2].

However, the lipids use in the diet and their effects on the health have been questioned. Effects of the different dietary fats on plasma lipids concentration were associated with diseases, such as coronary heart disease, the leading cause of death in the western world and in developing countries. A positive correlation between a high intake of saturated fat and thrombosis has been described though, a reduction in thrombotic tendency has been observed when polyunsaturated fatty acids (PUFAs) of the n-6 and n-3 series are incorporated [3].

Sunflower oil is rich in PUFAs, especially linoleic acid (C18:2ω6), and in monounsaturated fatty acid (MUFA), specifically oleic acid (C18:1ω9). Some studies indicate that linoleic acid deficiency leads to reduction in the reproductive functions, while others found an increase in the proportion ω6:ω3 in astenozoospermics and oligozoospermics human samples [4-7].

Mammalian spermatozoa are characterized by their phospholipids composition, which contains large amounts of PUFAs, particularly docosahexaenoic acid (C22:6ω3), docosapentaenoic acid (C22:5ω3) and arachidonic acid (C20:4ω6), and the later being a linoleic acid derivative. The main MUFAs are oleic acid, another component of the sunflower oil, and palmitoleic acid (C16:1ω7), whereas the main saturated fatty acids (SFAs) are palmitic (C16:0) and estearic (C18:0) acids. These fatty acids seem to play an important role in the quality of spermatozoa and in the fertilization processes in mammals [6,8,9].

Phospholipids and their classes are correlated with sex and reproductive stage. Phosphatidylcholine is the predominant phospholipid class in spermatozoa, whereas sphingomyelin is the major in the seminal plasma. In semen, the composition of sperm membrane lipids is dependent on the exchanges of these molecules between spermatozoa and the seminal plasma [10-12].

Furthermore, lipids are known to have a major influence on the structure and function of spermatozoa. The quantity and the composition of these lipids in the plasma membrane are known to have a significant effect upon the physicochemical properties and in turn, upon functional characteristics of the spermatozoa, such as membrane fluidity, spermatozoa viability, motility, maturity, acrosome reaction and sperm-oocyte II fusion. Changes in

membrane lipid composition are convenient means of altering membrane physical properties [6,11,13,14].

Few studies relate dietary fats and membrane spermatozoa status. Thus, the aim of this study was to evaluate the effect of sunflower oil supplementation on plasma, seminal lipid profile, and on the functional integrity sperm membrane, in goats (*Capra hircus*) from semiarid region of the Northeastern, Brazil, since these small ruminants are very important for the economy of this region, as well as for many other countries.

2. Materials and Methods

2.1. Location

The study was carried out in Patos, Paraíba - Brazil. This city is located 249.09 m above sea level at 07 ^{o} 01 ' S and 37 ^{o} 16 ' W. Its climate is classified as hot and semiarid. During the months of the experimental period, from July to October 2003, the minimum and maximum temperatures averaged 27.7 ^{o}C and 37.2 ^{o}C, respectively. This year's humidity and temperature averaged 53 % and 27.8 ^{o}C, respectively, and the pluviometric precipitation totalled 652.7 mm.

2.2. Animals and Diets

27 Anglo-Nubian crossbred mature male goats (*Capra hircus*), aged 2 to 4 years-old and with mean weight of 54.04 ± 8.67 Kg, were selected and divided in 3 groups of 9 animals, and kept for 60 days into individual stalls, at environmental temperature. One group received 2 % sunflower oil supplementation, while other received 4 % sunflower oil and the third group was submitted to diet containing 6 % sunflower oil addition. The diets were adjusted in order to attain the maintenance requirements stated by AFRC [15], and they were offered in two equal portions corresponding to 3 % of body weight, to all groups in the same time of the day. Fresh water was freely available. It is worthwhile mentioning that sunflower oil contains 68.5 % of C18:2ω6, 21.7 % of C18:1ω9, 5.5 % of C16:0, 3.6 % of C18:0, and just traces of C18:3ω3, C14:0 and C20:0, as described by Van Soest [4] .

2.3. Collection of plasma and semen samples

Plasma and semen were obtained before (0 d) the animals having received the supplementations, 30 days (30 d) and 60 days (60 d) of diet intake supplemented with sunflower oil. Blood samples were collected by jugular venipuncture into vacuum tubes (Vacuette, Brazil) containing ethylenediaminetetraacetic (EDTA) as anticoagulant, and they were immediately centrifuged at 2500 xg for 15 minutes for plasma collection. Aliquots of plasma were stored at –20 ^{o}C for later biochemical determinations. Semen samples were collected into a graduate tube using an artificial vagina and one oestrogenized teaser doe. The semen was kept in a tube in water bath at 37 ^{o}C, and immediately used.

2.4. Lipid Analysis

Lipids were extracted from plasma and semen samples as described by Folch [16], with chloroform : methanol (2 : 1, by volume) (Merck, Germany). Total cholesterol (TC) and total phospholids (PL) levels, phosphatidylcholine (PC), sphingomyelin (SPH), lysophosphatidylcholine (LPC) and phosphatidylethanolamine (PE) were either in plasma and in semen, but triglycerides (TG) levels were determined only in plasma, and some lipid classes, such as phosphatidic acid (PA), phosphatidylserine (PS), glycosphingolipids (GSL), glycerophosphorylcholine (GPC) and phosphatidylinositol (PI) percentages were determined only in semen. TC and TG were assayed by enzymatic methods (Labtest, Brazil), PL and their classes were by Bartlett's method and GSL were according to NELSON [17,18,19]. The isolation of the phospholipid subclasses was by one-dimensional, for the plasma, and by two-dimensional thin-layer chromatography (TLC), for the semen. Two solvent systems were used, one with chloroform : methanol : aqueous ammonia (65 : 35 : 5, by volume) (Merck, Germany), for the first dimension of two-dimensional TLC, and other with chloroform : acetone : methanol : acetic acid : water (50 : 20 : 10 : 10 : 5, by volume) (Merck, Germany), for the second dimension of two-dimensional and for the one-dimensional.

2.5. Hypoosmotic Swelling Test and Sperm Concentration

To the hypoosmotic swelling test, 100 µl aliquots of semen were added to a hypoosmotic solution (150 mOsmol) and were kept at 37 ^{o}C by 30 minutes. Spermatozoa were observed in a phase contrast microscope (Zeiss, Germany), and at least 200 sperm cells had to be counted. The results were expressed in percentage of spermatozoa reactive to the medium. Sperm cell concentration was measured by using a haemocitometer (Assistent, Germany).

2.6. Statistical Analysis

Data were analysed with Stat View 50 Software for Windows (SAS, 1998). Differences between the same animals on the 0 d, 30 d and 60 d were accessed by Multivariate Analysis of Variance (MANOVA) followed by Paired Student t Test, while the differences between the groups (2 %, 4 % and 6 % sunflower oil) were accessed by Unpaired Student t Test. Differences were considered significant when $p < 0.05$.

3. Results

As shown in Figure 1, by using 2 %, 4 % or 6 % sunflower oil on goat diet, plasma TC level was significantly increased in the animals in 60 d when compared to the same animals in 0 d. Nevertheless, semen TC concentration increased significantly with the use of 2 % and 6 % sunflower oil along this period, but it was essentially unchanged by 4 % fat administration.

No changes occurred on plasma TG levels in goats fed different concentrations of sunflower oil, during the time of the experiment. However, the plasma PL level was significantly higher, after 60 d, in goats fed 6 % (4.25 ± 0.25 mmol/L) sunflower oil than in those having 2 % (3.4 ± 0.2 mmol/L) fat addition. In the semen, it was observed a significative increase of PL level in the caprines fed 2 % sunflower oil when compared to the same animals on 0 d diet supplementation (6.0 ± 0.3 mmol/L) with 30 d (7.5 ± 0.8 mmol/L) and with 60 d (8.4 ± 1.0 mmol/L).

The composition of plasma and semen phospholipid subclasses of goats was also affected by the fat supplement. The percentage of PC was higher in both, plasma and semen. In the later, the amount of SPH and GPC were similar, and they were followed by LPC and PE, whose composition did not differ from each other. The amount of plasma PC was followed by LPC, SPH and PE, as shown in Figure 2. Although the relative values of LPC decreased in all the analysed samples, it achieved statistical significance in the plasma of goats in 60 d with 2 % sunflower oil, whilst in semen LPC was significantly decrease in 60 d with 4 % and 6 % fat supplementation, as shown in Figure 3.

When the groups were compared in 60 d on sunflower oil supplementation, the percentage of plasma PC values was significantly decreased in goats fed 4 % lipid supplementation. This decrease in PC was accompanied by a significant increase in SPH values, as seen in the Figure 4.

However, in the semen, it was found a significant increase of PC in the caprines fed 2 % sunflower oil supplementation, whilst SPH was significantly increased in goats receiving 4 % fat, as demonstrated in Figure 5.

Figure 6 makes evident the higher relative values of GSL in the semen of these small ruminants under 4% fat addition in their feeding.

The other plasma and seminal lipids determined in this work, as mentioned in materials and methods, did not undergo statistical significant changes in goats fed sunflower oil different concentrations, during all time of the experiment (data not shown).

Regarding to sunflower oil supplementation a positive effect was seen on the status of spermatozoa membrane and on spermatogenic process, as demonstrated in Figure 7. A significative increase in the percentage of reactive spermatozoa to the hypoosmotic swelling medium was found at 60 d, and the number of spermatozoa also increased during the experiment with the use of the differents sunflower oil percentages in the diets of the animals.

4. Discussion

Specific effects occasioned by different types and quantity of fatty acids in the diet on plasma and spermatozoa membrane fatty acid composition and on spermatozoa properties are complex and difficult to evaluate.

In sexually mature ruminants, nutrition is one of the primordial factors and the responses to it can to be divided into short-term effects, that act mainly on the neuroendocrine system controlling testicular activity, and long-term effects, that act on testicular growth and sperm production. Thus the effect of some polyunsaturated fatty acid rich diets on the plasma and seminal lipid profile were correlated to the status of the spermatic membrane along 60 days, since previous studies [20], reported that periods smaller than 30 days may be insufficient, considering that maturation of spermatozoa occur in about 45 days.

The plasma levels of total cholesterol were increased, in all groups of lipid supplementation, but no changes occurred in the semen of 4% sunflower oil fed animals This in accordance to a study, which reports serum cholesterol of ruminants animals to rise when supplemental fat is given in a way that ensures the absorption of long-chain fatty acids [21], and to a number of other studies that have shown a rise in cholesterol concentration in the blood plasma but not always in the tissues of fat supplemented ruminants. As ruminants commonly supply all of their cholesterol from endogenous biosynthesis, it is reasonable to suppose that the fat-induced cholesterol increase is due to either an elevated “de novo” synthesis of cholesterol and/or a decreased fecal excretion of cholesterol or bile acids.

It was also showed the small intestine, and especially its most proximal part, as the only organ to present a fat-induced enhancement of sterolgenesis [21] . It seems highly likely that increased cholesterolgenesis in the small intestine is a major factor in the development of the hypercholesterolemia, since earlier *in vitro* studies revealed a fat-induced suppression of hepatic cholesterolgenesis. This reduction supports the fact that fat-induced hypercholesterolemia is reflected by risen intestinal cholesterolgenesis. The effect of 2%, 4% and 6% sunflower oil diets was an increase in the blood circulation of cholesterol but little change in its turnover. It has been described [22] a significant increase in serum total cholesterol value in the ω6 polyunsaturated fatty acids rich diet, with 9% of sunflower oil diet accessed after 50 weeks.

It is related [23] that cholesterol is abundant in the sperm cell membrane and is an essential constituent of mammalian semen. Cholesterol plays an important role in the fertilization capacity of these cells, like a critical role in regulating the molecular packing, permeability, stability, and fusogenicity of sperm cells membranes. Membrane cholesterol content varies along the way from the testis, through the epididymis, to the female reproductive tract, where an efflux of cholesterol occurs in preparation to the sperm-egg interaction, as previously mentioned [8].

On the other hand, in a work with cholesterol incorporation on equine sperm membrane, it was not verified enhance in fertility [24]. It was also observed [25] the influence of dietary lipids on microsomal membranes and they reported an increase in membrane fluidity and a decrease in membrane cholesterol content when a standard diet was supplemented with 10% sunflower seed. This can justify the increase in the percentage of reactive spermatozoa to the hypoosmotic swelling test in goats with 4% sunflower oil supplementation, independent of total cholesterol levels, in the present study.

It has been noted that the high fat diets significantly raised plasma total cholesterol, triglycerides and phospholipids [20], but in other study [22], triglycerides values did not exhibit any remarkable changes.

In this work, there was not found significative change in triglycerides plasma levels. Others [26,27] have reported that supplemental dietary fat increased blood cholesterol and nonesterified fatty acids but caused inconsistent changes in blood triglycerides in ruminants.

Regarding to the total phospholipids plasma concentrations, the results obtained in this work show an increase in these lipids in goats fed with 6% sunflower oil addition after 60 days in accordance to a work previously mentioned [20]. However, studying hyperlipidemic effects on captive and wild alligators [28], found no significant differences in phospholipids levels between the two groups, although linoleic acid has significantly higher in captives than in wild alligators. In the present study, phospholipids also had a significant increase in the semen in the group receiving 2% fat addition. The increase of phospholipids levels with time can be explained by the improved flux of fatty acids of the diet.

In all species, phospholipids are the major lipid components of spermatozoa, and they contain large amounts of ω6 polyunsaturated fatty acid is an essential serie of fatty acids, since they cannot be synthesized in vertebrates and must be provided by the diet. In a study with chicken semen [29], it has been suggested that the lipid and fatty acid composition of spermatozoa may be important determinants of fertility.

In this work, the same phospholipids classes profile was observed in plasma blood and in semen. Phosphatidylcholine was the major phospholipid followed by sphingomyelin, in agreement with many works [8,9,11,30], even though the range of percentages vary so much between them. Lower values of phospholipids classes in semen were encountered in this study than in others, due possibly to the methodology, since, here, it was used two-dimensional thin-layer chromatography, which allows the separation of a large number of classes, decreasing thus their relative values.

But, these values are still kept in the range mentioned by other authors [8], except for the PC%, which was considered smaller than the average, since GPC% was also determined. GPC is a derivative of PC when there is an addition of phospholipids to sperm during epididymal transit with degradation of these phospholipids by sperm to yield GPC or degradation of phospholipids by epididymal epithelial cells with excretion of GPC into the duct lumen [31].

It was also noted [9,32] that not all phospholipid classes are detected with the same sensitivity and that especially the contribution of PC and LPC to the total phospholipid content is overestimated since other phospholipids are less sensitively detected. Thus, with due regard to the fact that the compositions of sperm cells membranes differ across animal species and sometimes between studies of the same species, the results presented here fall within the range of values reported in the literature.

The values of LPC decreased in all the analysed samples, after the use of sunflower oil supplementation. These results are in agreement with those found in lecithin-cholesterol acyltransferase (LCAT) deficiency, and could be attributed to the fact that cholesterol is not being esterified with C18:2ω6 from PC, as mentioned by the others authors [14], which related the effect of diet on membrane lipid composition and morphology of rat erythrocytes. They suggested that a higher availability of C18:2ω6 sunflower oil-fed animals occasioned an increased PC turnover. However, it has been reported [33] that the

esterification process of LCAT was shown to have a high specificity for C18:2 under the conditions of the experiment, which occurred *in vitro*.

But, this same study [33] also showed that the LCAT present in bovine plasma was completely discriminatory against the large concentrations of C18:1 esterified in the sn-2 position of phosphatidylcholine, and it has been described [34,35] that LCAT is stereospecific. The decrease of the LPC, can be explained since, in ruminants, polyunsaturated fatty acids are hydrogenated by microbes, in the rumen, to more saturated end products [36,37]. According to the other study [7], the extent of these lipid transformations is neither constant nor complete, and trans fatty acids can appear in great quantity and be incorporated to the phospholipids.

The seminal phospholipid composition is very important, because phospholipids are major structural components of membranes. Data reported that the majority of long chain metabolites of linoleic acid is present in human spermatozoa, and that oleic acid and its metabolites were detected in human spermatozoa, and in bovine and swine tests [6]. It was observed [38], studying the effect of essential fatty acid deficiency on lipids of rat Sertoli and germinal cells, that in some rats there were decreases in linoleic and arachidonic acids and that feeding a lipid diet to these rats reversed the changes in fatty composition of both Sertoli and germinal cells at the times studied.

However, in ruminants [36,39], addition of lipid to the diet of these animals causes only transient increases in ruminal abomasal polyunsaturates and that enhancement of unsaturated fatty acid flow to the duodenum is very limited. Others authors [40] fed blended animal-vegetable fat (59% unsaturated) to dairy cows and noted that intake of linoleic acid increased from 171 to 296 g/d, but duodenal flow increased only from 45 to 54 g/d.

Then, some dietary unsaturates escape biohydrogenation and when high amounts of unprotected polyunsaturates are fed to ruminants, absorption of these acids increase only slightly, still according to early mentioned [36], and the sunflower oil administrated in the present experiment, was an unprotected form.

The old adage “what you eat is what you get” is incorrect in the case of dietary lipids and ruminants animals [37], and this is of special importance to include lipid metabolism in dynamic models of ruminant digestion. The knowledge of lipid digestion and metabolism is

advancing and challenge is to effectively apply this knowledge in the feeding and management of reproductive ruminants.

These facts may explain the significative differences occurred into each group, for example, the statistically significant elevation of PC% in the semen in goats fed with 2% sunflower oil but not in those receiving 6%, although this group values also increased with time after the diet.

Regarding to the status of spermatozoa membrane, the hypoosmotic swelling test showed that there was an enhancement in the functional integrity of the membrane in all groups, since there was an increase in the percentage of reactive spermatozoa in the hypoosmotic solution, or swollen spermatozoa. Sperm cells with membrane damage do not swell, therefore the tail does not coil. So, with the sunflower oil supplementation, there has been changes in the goats' sperm cells membranes, important for normal spermatozoa metabolism and function, indicating a better semen quality and fertilization capacity.

This can be justified by the increase of the relative values of PC in the semen of the animals under 2% sunflower oil supplementation and by the increase of the relative values of SPH and GSL in the total semen of the goats under the diet with 4% of lipid supplementation. The addition of 6% sunflower oil led to an increase in relative values either of PC and SPH, although they were not statistically significant. The 6% diet may have favoured a escape of fatty acids to biohydrogenation. However, it also was found an increase of swollen spermatozoa, which can indicate that the fatty acid individual composition needs to be investigated too.

But, the importance of the classes of phospholipids and their relations with sex and reproductive stage cannot be denied. The cell membrane is an interface for communication between the inner and outer cell medium and each phospholipid class plays a different role in the regulation of the membrane activity. Thus, variations in phospholipids moieties in specific organs are a direct reflection of regulation in individual cell activity depending on a particular physiology, as reported by some authors [12].

Accumulation of polar lipids during vitellogenesis, especially PC and PE, has been already shown in the ovary of benthic decapods [41], and this seems to indicate high lipid metabolic activity and transformation during gonad maturation. In euphasiids, it has been observed [42] a correlation between the increase of total lipids and the increase in PC,

leading to the hypothesis that PC in semen acts as a source of essential nutrients, such as organic phosphorus, choline and fatty acids to the spermatozoa. Due to its great proportion, phosphatidylcholine can be an important source of essential PUFAs, like linoleic acid, as earlier mentioned [12], that also reported a study with incorporation of radiolabelled linoleic acid in *Meganyctiphanes norvegica*.

It has been related PC deficiency with decreased membrane fluidity, stressing the importance of this phospholipid class to spermatozoa [34], and that PC also may serve as a regulation component for the transmission of the membrane action potential [43].

Many authors [44]also suggest that factors of the seminal fluid are important for the sperm acquisition of fertility, and SPH is a major phospholipid in seminal plasma, besides be a essential structural and functional component of membrane, as reported [10]. The outer layer of goat sperm cell plasma membrane is rich in PC and SPH.

In the present research, the higher values of GSL in total semen of the caprines under 4% diet were also evident. It has been found [45] that glycolipids are concentrated in the apical region of spermatozoa, in freshly ejaculated sperm cells. It is likely that glycolipids prevent the early acrosome reaction by stabilizing the lipid lamellar bilayer of the plasma membrane.

In order to be capable of fertilizing the oocyte II, spermatozoa must undergo the acrosome reaction and the capacitation process. During the capacitation process, seminolipids migrate into equatorial region of reaction, reflecting an important step in the capacitation event, which may be essential for mammalian fertilization.

Authors [46] has been suggested that low fluidity could explain the failure of fertilization by sperm of men with pathological semen characteristics. Moreover, an increased local polarity and fluidity of the sperm cell membrane is associated with the acquisition of its fertilizing ability [47].

Thus, it suggests that what happened in these small ruminants under diet containing sunflower oil was a better fluidity of sperm cell membrane, demonstrated by the increase in the percentage of swollen spermatozoa in the hypoosmotic swelling test, justified by the possible increase of unsaturated fatty acids in the phospholipids, especially in the PC and SPH classes.

The number of spermatozoa in the ejaculated increased too, after sunflower oil supplementation, emphasizing, as cited previously [38], that essential fatty acids may have important consequences on Sertoli cells, playing a significant role in the spermatogenic process. According to the other study [48], all mammalian reproductive processes will ultimately be determined by nutrient availability, from gametogenesis to lactation.

Thus, the data of this work suggest that supplementing the feeding with 2% sunflower already is enough to promote an enhance in the reproductive performance of males goats and, although metabolic studies would be needed, the present study gives new insights of possible major pathways of lipids involved in reproduction of goats.

ACKNOWLEDGEMENTS

This research was supported by the Conselho Nacional de Pesquisas e Desenvolvimento Tecnológico – CNPq, Brazil (Grant 521340/96-9). The authors thankfully acknowledge the facilities put available by the Veterinary Hospital of the Federal University of Campina Grande.

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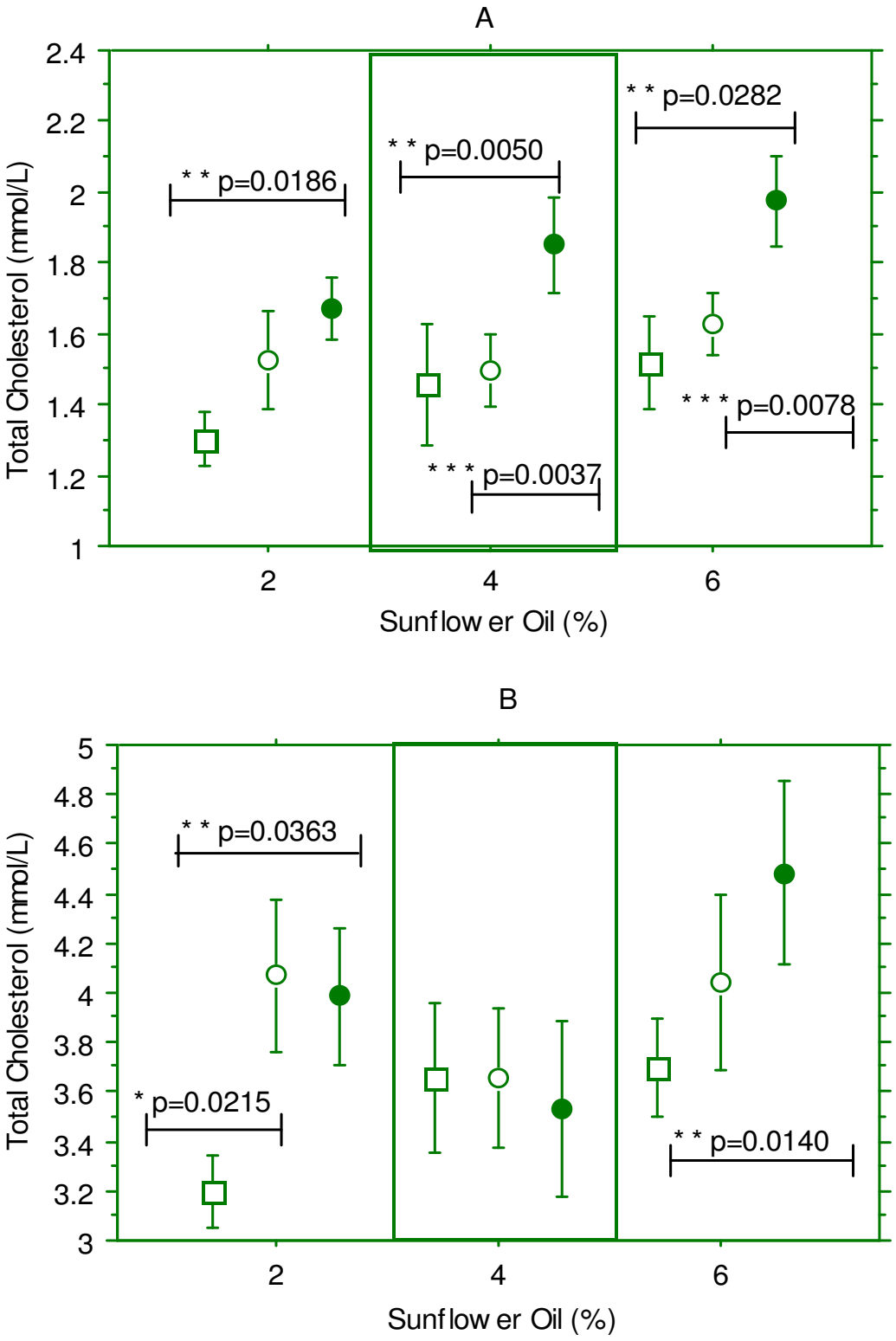

Figure 1. Comparison levels of total cholesterol in goats fed 2 %, 4 % and 6 % sunflower oil supplementation. Data are means ± SEM for plasma (A) and semen (B) before – 0 d (□) sunflower oil supplementation, 30 days – 30 d (○) and 60 days – 60 d (●) on sunflower oil supplementation.* Significance level found between 0 d and 30 d. * * Significance level found between 0 d and 60 d. * * * Significance level between 30 d and 60 d.

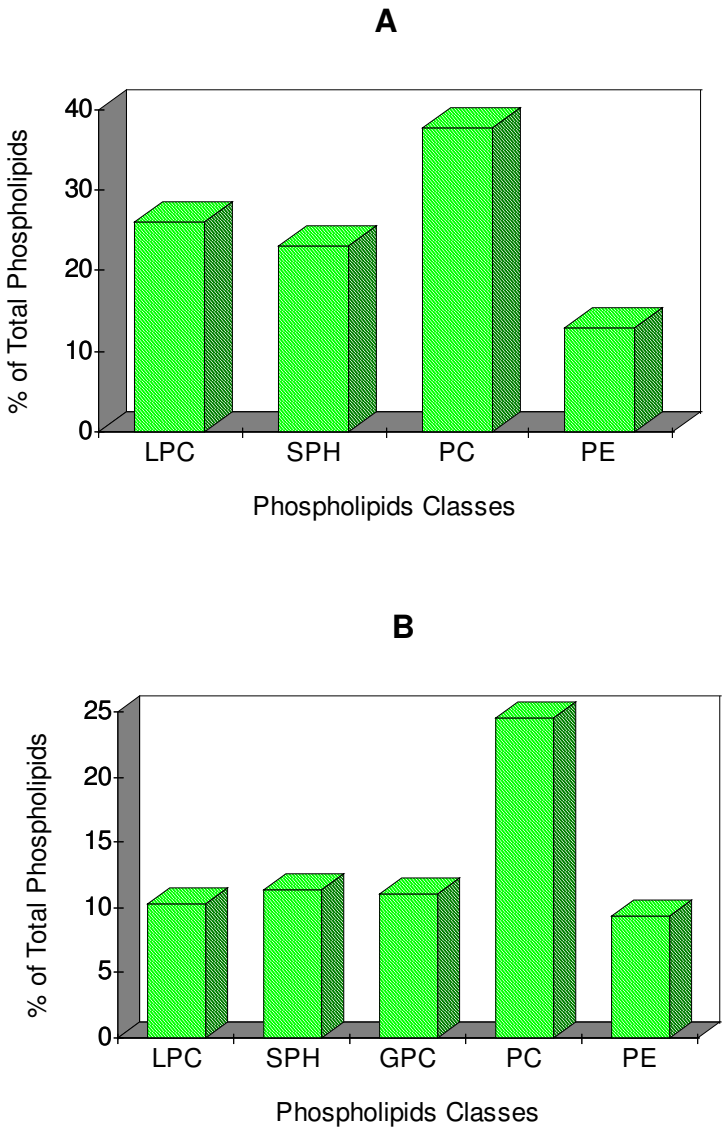

Figure 2. Relative composition of phospholipids classes in plasma and semen from goats. A, plasma phospholipids were isolated by one-dimension TLC. B, semen phospholipids were isolated by two-dimension TLC.

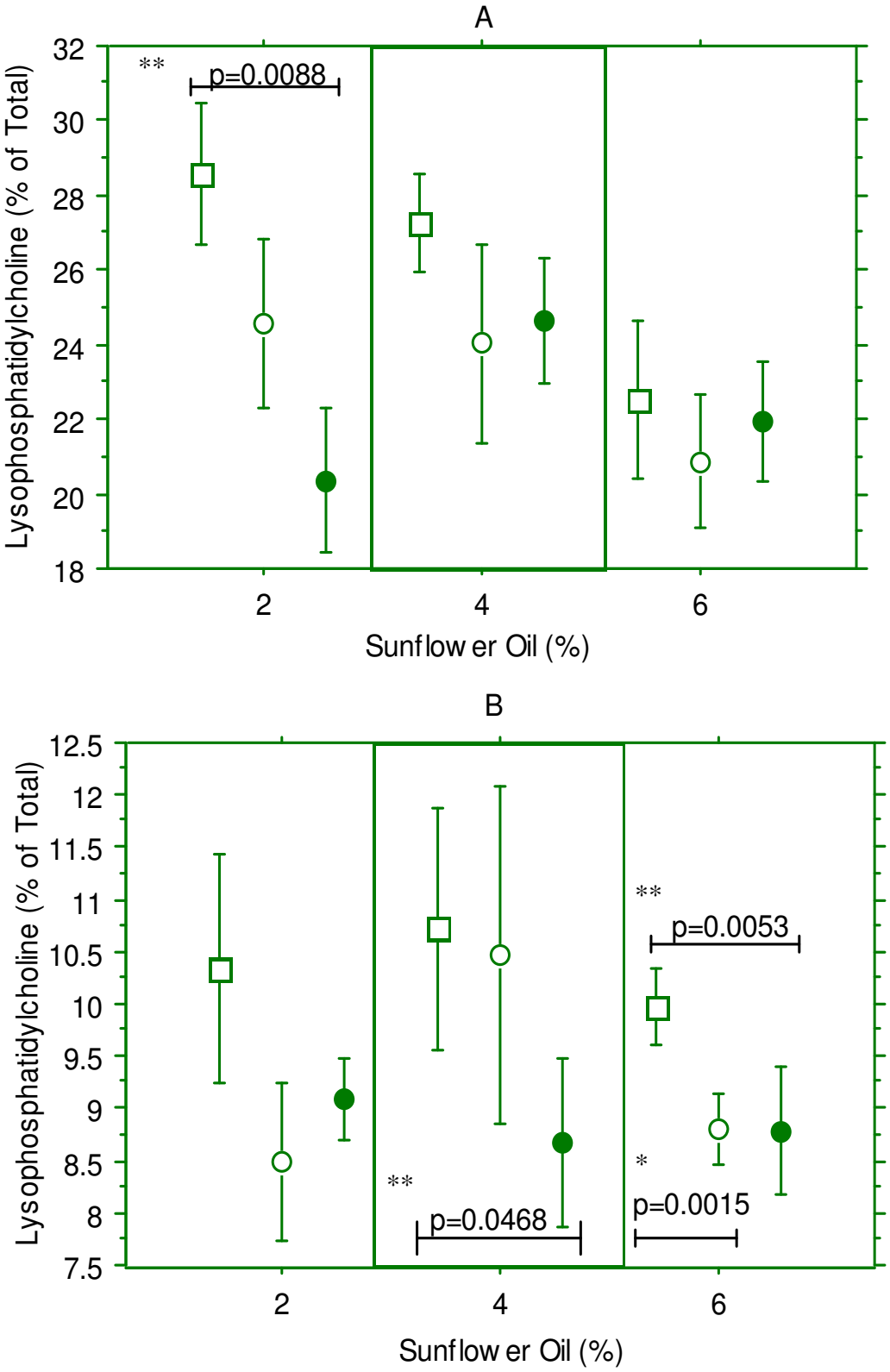

Figure 3. Percentage of lysophosphatidylcholine in goats fed 2 %, 4 % and 6 % sunflower oil supplementation. Data are means ± SEM, for goat plasma (A) and semen (B), before – 0 d (□) sunflower oil supplementation, 30 days – 30 d (○) and 60 days – 60 d (●) on sunflower oil supplementation. * Significance level found between 0 d and 30 d. * * Significance level found between 0 d and 60 d.

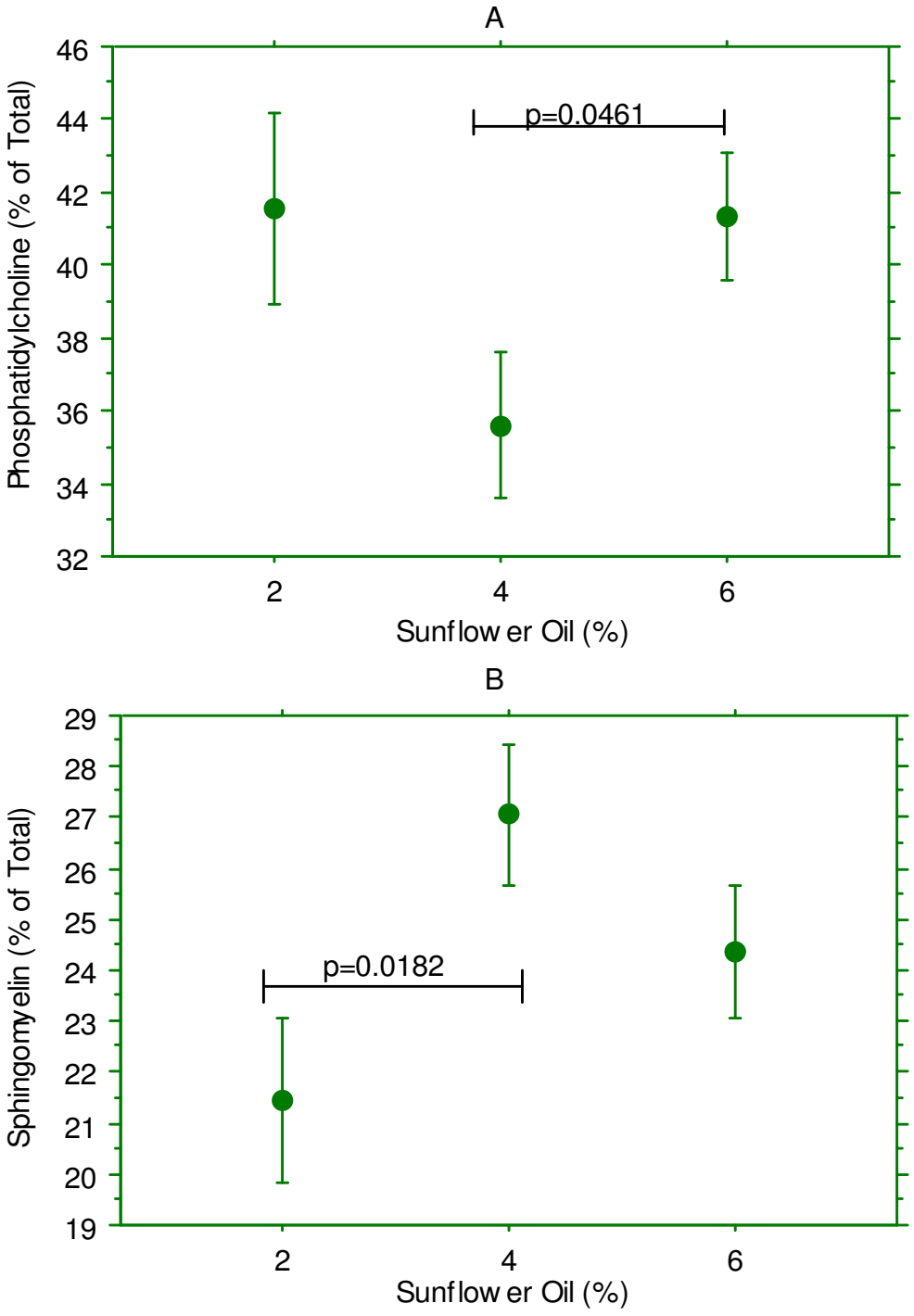

Figure 4. Percentage of plasma phosphatidylcholine (A) and sphingomyelin (B) from goats with 60 days on sunflower oil supplementation. Data are means ± SEM.

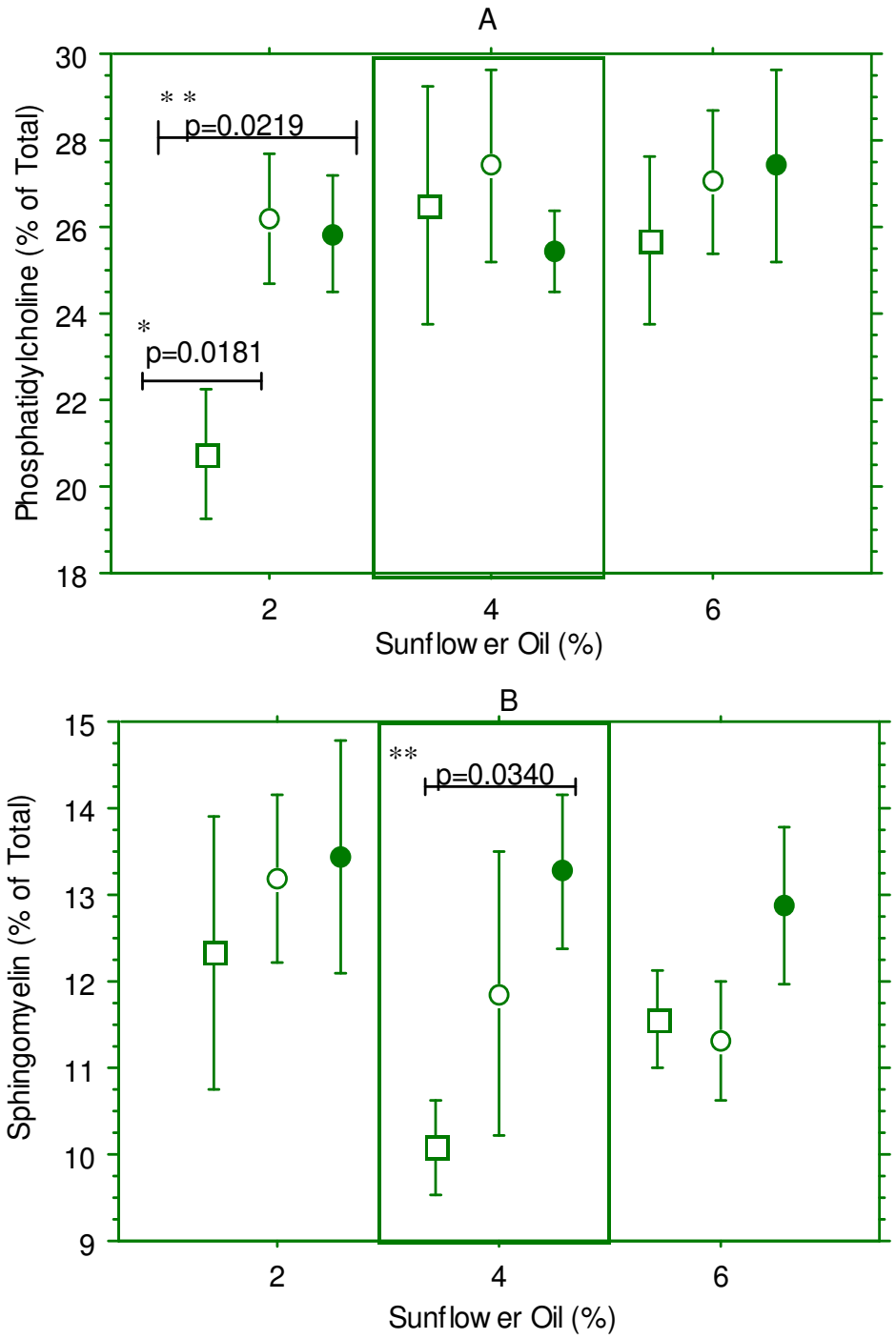

Figure 5. Percentage of phosphatidylcholine and sphingomyelin in semen of goats fed 2 %, 4 % and 6 % sunflower oil supplementation. Data are means ± SEM, for phosphatidylcholine (A) and sphingomyelin (B), before – 0 d (□) sunflower oil supplementation, 30 days – 30 d (○) and 60 days – 60 d (●) on sunflower oil supplementation. * Significance level found between 0 d and 30 d. * * Significance level found between 0 d and 60 d.

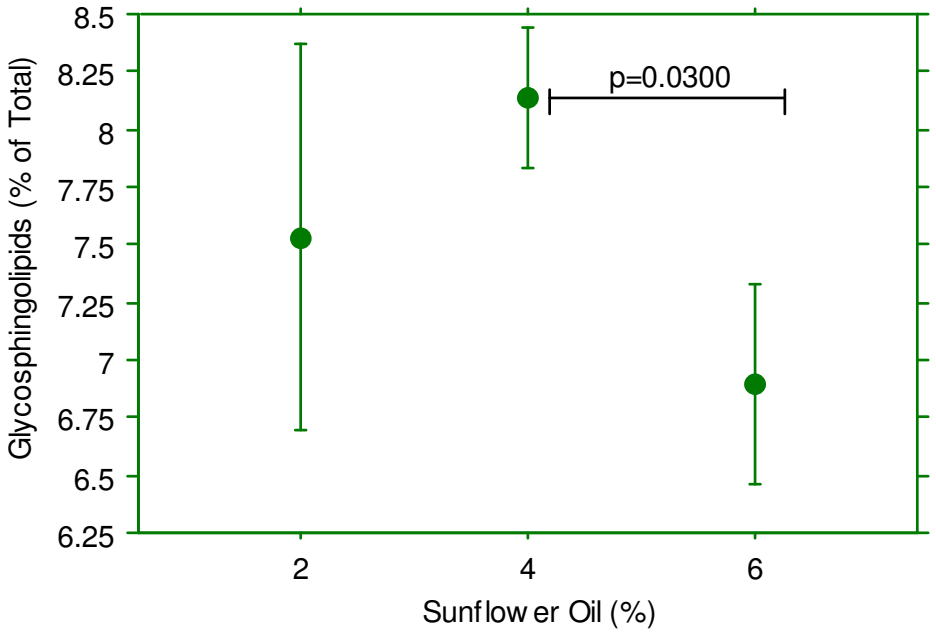

Figure 6. Percentage of semen glycosphingolipids in goats on sunflower oil supplementation for 60 days. Data are means ± SEM.

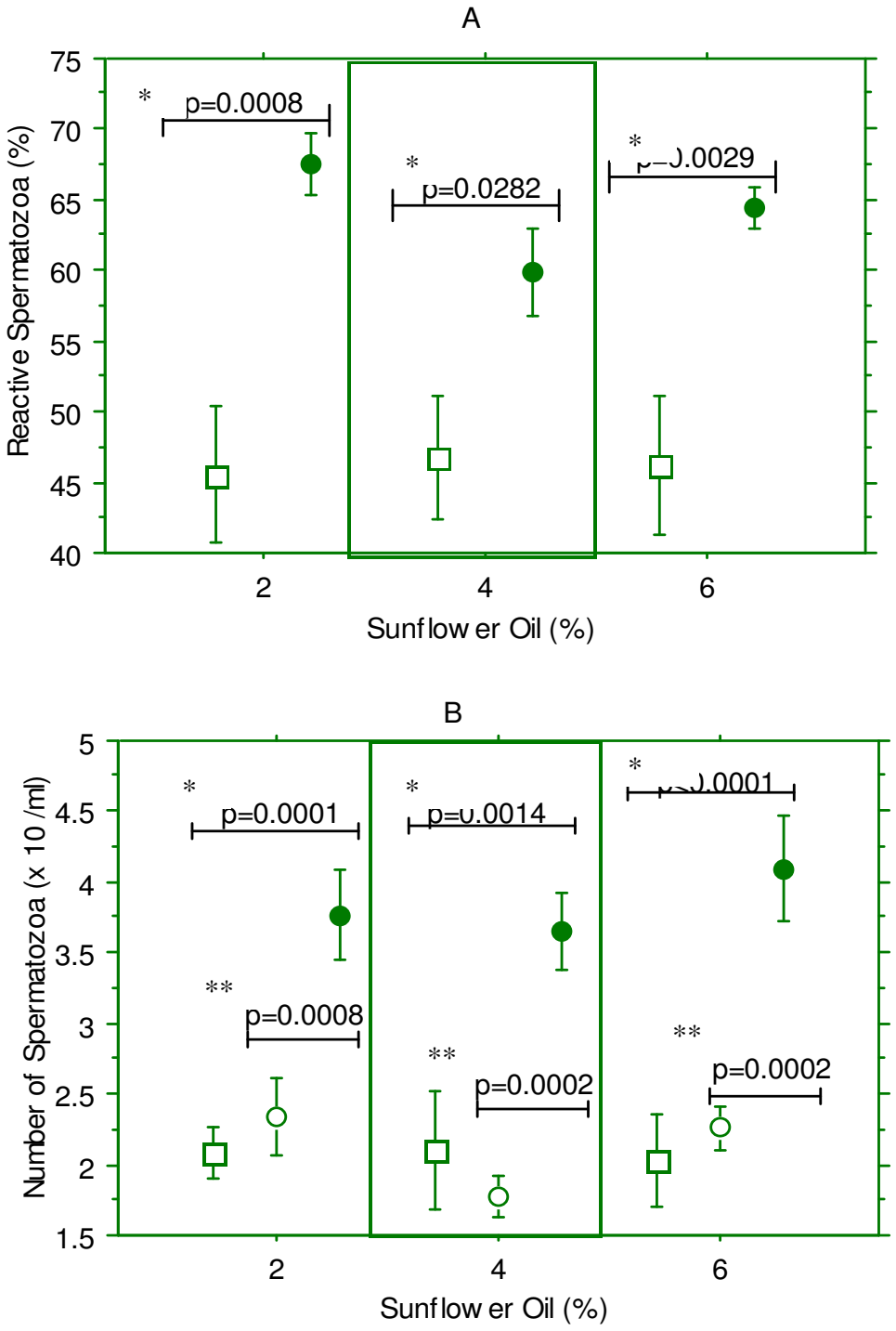

Figure 7. Effect of sunflower oil supplementation effect on fertility and on spermatogenesis in semen of goats. (A) Fertility accessed by percentage of reactive spermatozoa to the hypoosmotic swelling test. (B) Number of spermatozoa (x 10^9/ml). Data are means ± SEM, before – 0 d (□) sunflower oil supplementation, 30 days – 30 d (○) and 60 days – 60 d (●) on sunflower oil supplementation. * Significance level found between 0 d and 60 d. * * Significance level found between 30 d and 60 d.

6. CONCLUSÕES

- O perfil lipídico plasmático e seminal de caprinos machos sofreu variação com o uso de suplementação com 2 %, 4 % e 6 % de óleo de girassol, mas nem sempre alterações nos lipídios plasmáticos refletiram alterações no sêmen, no período de 60 dias;

- Suplementação alimentar com óleo de girassol, nas concentrações de 2 %, 4 % e 6 %, aumentou o processo espermatogênico e provocou uma melhora na fertilidade de caprinos machos, devido a uma maior integridade funcional da membrana espermática, relacionada com alterações de algumas classes lipídicas, dependentes de ácidos graxos insaturados em sua composição, como fosfatidilcolina, esfingomielina e glicoesfingolipídios.

- Suplementação de dieta com 2 % de óleo de girassol foi suficiente para promover melhora do desempenho reprodutivo de machos caprinos.

7. PERSPECTIVAS

- Investigação da composição individual dos ácidos graxos dos espermatozóides a fim de esclarecer melhor o efeito de dietas suplementadas com ácido linoléico no metabolismo lipídico ruminal desses animais.

8. ANEXOS

TRABALHOS ENVIADOS PARA CONGRESSOS

GUIDE FOR AUTHORS

TRABALHOS ENVIADOS PARA CONGRESSOS

XXXIV REUNIÃO ANULA DA SOCIEDADE BRASILEIRA DE BIOQUÍMICA E BIOLOGIA MOLECULAR – SBBq , NA FORMA DE COMUNICAÇÃO ORAL

EFFECT OF SUNFLOWER OIL ON SEMEN CHOLESTEROL, PHOSPHOLIPIDS AND FERTILITY OF MALE CAPRINES

Santos, B. S. dos[1]; Azevedo, S. A.[2]; Pimenta Filho, A. A.[1]; Pereira, D. R.1; Azevedo Neto, J.[2]; Carvalho, V. C. O.[1]; Lima, V. L. M.[1]

[1]Departamento de Bioquímica – CCB, UFPE; [2]Departamento de Medicina Veterinária – CSTR, UFCG.

In semen, the composition of sperm membrane lipids is dependent on the exchanges of these molecules between spermatozoa and the seminal plasma. The quantity and the composition of the sperm cell membrane and semen lipids are known to have a significant effect upon the functional characteristics of this cell, such as viability, motility, membrane fluidity, acrosome reaction and sperm-oocyte II fusion. Changes in membrane lipid composition may alter membrane properties and function. Studies relating dietary fats and fertility are scanty. This study aims to evaluate the effect of 2%sunflower oil supplementation, rich in polyunsaturated fatty acids, especially linoleic acid (C18:2ω6), on seminal lipid profile and on the functional integrity of sperm membranein adult male goats (*Capra hircus*), since they are very important for the economy of many countries. Hypoosmotic swelling test (indicator of fertility), sperm cells and semen lipids were determined before and after 30 and 60 days on diet-supplement. Phospholipid subclasses were isolated by two-dimensional thin-layer chromatography. Total cholesterol was assayed by enzymatic method, and phospholipids by chemical method. Differences between the animals on the times were accessed by MANOVA. There was an increase in the total cholesterol, total phospholipids and phosphatidylcholineseminal values during the time of experiment. This was related to a significant increase in the percentage of reactive spermatozoa to the hypoosmotic solution, and in the sperm concentration when lipids were added to the diet. The results show that 2% sunflower oil supplementationto the capine diets is able to influence the fluidity of spermatozoa membrane, enhancing the fertility of these animals. Furthermore, the diet seems to have a significant role in the spermatogenic process. The improvement on the fluidity of spermatozoa membrane may be due to a better quality of its phospholipid component, especially phosphatidylcholine, having a good source of unsaturated fatty acids.

A REUNIÃO DA FEDERAÇÃO DA SOCIEDADE DE BIOLOGIA EXPERIMENTAL, 2005 – FESBE, 2005

DIET SUPPLEMENTATION WITH SUNFLOWER OIL IMPROVES THE SPERMATOGENESIS IN MALE GOATS (*Capra hircus*). SANTOS, B. S. dos, PEREIRA, D. R.*, PIMENTA FILHO, A. A., AZEVEDO, S. A., AZEVEDO NETO, J., CARVALHO, V. C. O., LIMA, V. L. M. Departamento de Bioquímica – CCB – Universidade Federal de Pernambuco (PE), Departamento de Medicina Veterinária – CSTR – Universidade Federal de Campina Grande (PB).

In sexually mature ruminants, nutrition is one of the primordial factors that act mainly on the neuroendocrine system controlling testicular activity. Some polyunsaturated fatty acid-rich diets may improve the sperm production. Thus, this study aims to evaluate the effect of diet supplementation with sunflower oil on the spermatogenic process in male goats, since this oil is rich in linoleic acid (C18:2ω6) which is an essential polyunsaturated fatty acid. Therefore, 27 mature male goats were selected, kept into individual stalls and split in 3 groups of 9 animals. Each group received one different fat supplemented diet (2%, 4% and 6% sunflower oil) for 60 days. Semen samples were collected into a graduate tube, using an artificial vagina and one oestrogenized teaser doe, before, and 30 and 60 days after these small ruminants having received the sunflower oil supplementations, and sperm cell concentration was measured by using a haemocitometer. Differences were considered significant when $p<0.05$. The number of spermatozoa increased significantly after 60 days on the three different sunflower oil-containing diet (about 81% in the group that received 2% sunflower oil supplementation; about 74% in the group that received 4%; and about 101% in the animals on diet supplementation with 6% sunflower oil), but no changes ocurred after 30 days. The results suggest that linoleic acid may have an important effect on Sertoli cells, and seems to play a significant role in the spermatogenic process, which needs about 7 weeks to complete the spermatozoa development.

SUPPORT BY CNPq

STATUS OF SPERMATOZOA MEMBRANE IS ENHANCED IN MALE CAPRINES ON SUNFLOWER OIL DIET SUPPLEMENTATION. SANTOS, B. S. dos, PEREIRA, D. R.*, PIMENTA FILHO, A. A., AZEVEDO, S. A., AZEVEDO NETO, J., CARVALHO, V. C. O., LIMA, V. L. M. Departamento de Bioquímica – CCB – Universidade Federal de Pernambuco (PE), Departamento de Medicina Veterinária – CSTR – Universidade Federal de Campina Grande (PB).

In Brazil, and in other countries, the production of caprine is a major economical activity. This work aims to investigate the effect of sunflower oil supplementation, rich in polyunsaturated fatty acids, especially linoleic acid (C18:2ω6), on the spermatozoa membrane status of caprines. In order to evaluate whether this supplementation is important for the fertility of these animals, since studies with male caprines are scanty. Thus, 27 adult Anglo-Nubian male caprines were selected and split in 3 groups of 9 animals. One group was submitted to diet containing 2% sunflower oil supplementation, while other received 4% and a third group received 6% sunflower oil addition. Semen samples were obtained before and 60 days after these caprines having received the sunflower oil supplementations, and the status of spermatozoa membrane was accessed by hypoosmotic swelling test. Therefore, 100µl aliquots of semen were added to a hypoosmotic solution (150mOsmol) and were kept at 37^{o}C by 30 minutes. Spermatozoa were observed in a phase contrast microscope, and at least 200 sperm cells had to be counted. Differences were considered significant when $p<0.05$. The percentage of reactive spermatozoa to hypoosmotic solution increased significantly 60 days after lipid supplementations to the diet (about 48% in the group that received 2% sunflower oil addition; about 28% in the group that received 4%; and about 39% in the animals on diet supplementation with 6% sunflower oil). These results suggest that supplementation with sunflower oil, containing essential fatty acids, to the animal diets was able to influence the fluidity of spermatozoa membrane. Thus, 2% sunflower oil supplementation already was enough to enhance the spermatozoa membrane status of male caprines.

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GUIDE FOR AUTHORS - THERIOGENOLOGY

Guide for Authors

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All submissions will be reviewed by at least 2 anonymous reviewers to evaluate them for originality, clear statement of a hypothesis, experimental design appropriate for the hypothesis, completeness of methods, thoughtfulness of the discussion and conclusions that are supported by data. Authors may name up to 5 potential reviewers and must provide complete contact information, however the Editor retains the right to assign different reviewers as deemed appropriate.

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Original Research Papers should report the results of original research. The material should not have been previously published elsewhere, except in a preliminary form.

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Circumstances relating to animal experimentation must meet the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organizations of Medical Sciences. These are obtainable from: Executive Secretary C.I.O.M.S., c/o WHO, Via Appia, CH-1211 Geneva 27, Switzerland or at the following URL: http://www.cioms.ch/frame_1985_texts_of_guidelines.htm .

Unnecessary cruelty in animal experimentation is not acceptable to the Editors of *Theriogenology*.

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page numbers; if necessary, one may refer to sections. Avoid excessive usage of italics to emphasize part of the text.

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Title Page (to include):

- Title, which should be clear, descriptive and not too long
- Running title, not more than 48 characters
- Names and professional affiliations of all author(s)
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Abstract: on a separate sheet not more than 250 words. The abstract should include the objective and main findings of the paper.

Five keywords (indexing terms)

Introduction: should acquaint the reader with the subject and justify the objective(s) of the research. There should be three parts to the introduction: first, a clear description of the nature and extent of the problem to be studied; second, a presentation of the pertinent research by others in the field of the study; and third, a statement of how the authors' study challenges, expands or improves the known material. The hypothesis or objective(s) addressed in the study must be clearly stated in the final paragraph.

Materials and Methods: must contain enough information to allow another scientist to duplicate the study. Materials should be named specifically, including the manufacturer, city and state or country where the equipment or supplies were obtained. Descriptions of animals should include species, breed, sex, and age as well as husbandry methods, climate, photoperiod and geographic location of the study. A logical description of the experimental methods should follow and should include an explanation of the experimental design. Here, it may be useful to prepare a table or schematic diagram to explain procedures, such as how the animals were divided into groups or how samples were obtained. The method of statistical evaluation must be stated, the treatment and response variables identified, and assignment of experimental units into groups specified.

Results: must contain sufficient information to fully describe the outcome of the research. The use of tables and figures is encouraged, but use text to emphasize important points, to connect results with one

another, and to restate the trend of the idea (the objective already mentioned in the INTRODUCTION). Tables and figures must contain enough information within them and in their respective titles or legends to be understandable without referring to the text.
Discussion: contains an explanation of the meaning of the results. The principles, relationships, and general truths shown by the results should be presented without retelling the results if at all possible. Exceptions or lack of correlation should be pointed out and unsettled points defined. Agreement or disagreement with previous work should be shown. The theoretical or practical implications of the work should be discussed. Finally, the major conclusions and implications should be stated in a brief paragraph.
Acknowledgements: and any additional information concerning research grants, etc.

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Connor EE, Ashwell MS, Dahl GE. Characterization and expression of the bovine growth hormone-releasing hormone (GHRH) receptor. Domest Anim Endocrinol 2002;22:189-200.

b. *For books*

Betteridge KJ. Embryo Transfer. In: Reproduction in Domesticated Animals, King GJ (Ed.), World Animal Science B9, Elsevier Science B.V., 1993, pp. 413-418.

c. *For multi-author books*

Van Zutphen LFM, Baumans V, Beynen AC. Principles of Laboratory Animal Science, Revised Edition. Elsevier Science B.V., 2001.

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5. In the case of publications in any language other than English, the original title is to be retained. However, the titles of publications in non-Latin alphabets should be transliterated, and a notation such as "(in Russian)" or "(in Greek, with English abstract)" should be added.
6. Work accepted for publication but not yet published should be referred to as "in press".
7. References concerning unpublished data and "personal communications" should not be cited in the reference list but may be mentioned in the text.

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The following words, phrases, abbreviations, and stylistic conventions are to be used when writing the manuscript.

- Avoid the word "injected," (e.g., "Cows were injected with PGF2 ") but include the generic name, proprietary name, dosage and route of administration (e.g., "Cows were treated with prostaglandinF2 [Lutalyse 25 mg im]").

Use the following expressions:

- palpated per rectum, not by rectal palpation.
- nucleus transfer, not nuclear transplant.
- estrus (noun) synchronization, but, estrous (adjective) behavior.
- 120 to 125, not 120-125. treatment by period, not treatment x period.
- gravity: 100 x g, but, magnification: x 100.
- identification number of an animal: No. 10, but, (number of) 30 animals: n = 30.
- day 3, but, 3 days (3 d).

Standard definitions:

- Oogonium: Female gamete before meiosis.
- Oocyte, primary: Female gamete from onset of the first maturation division (meiosis) until extrusion of the first polar body.
- Oocyte secondary: Female gamete from onset of second meiosis until extrusion of the second polar body.

- Ovum: Female gamete from the end of both meiotic divisions until the union of the male and female pronuclei. Note, this definition differs from the commonly use of ovum as general term for any female gamete.

- Germinal vesicle: Nucleus of the ovum.

- Zygote: A fertilized ovum, from the fusion of the male and female gamete until completion of the first cleavage.

- Embryo: A conceptus from 2-cells until after cell migration and differentiation are largely completed.

- Fetus: A conceptus after most of organogenesis is completed and it is primarily growing in size.

- Conceptus: An embryo or fetus with all its membranes and accessory structures.

- Abortion: Expulsion of a conceptus incapable of independent life.

- Premature parturition: Expulsion before full term of a conceptus capable of independent life.

- Stillbirth: Do not use this term, use fetal death or abortion.

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Never use an abbreviation to start a sentence. Some abbreviations may be used anywhere else, including the manuscript's title and figure and table titles and legends, without definition; others may not be used in the title, but may be used in the text without definition. In general, abbreviations must be defined when used for the first time (this may be avoided in the ABSTRACT if necessary to conserve space). To make reading the paper more pleasant, avoid using abbreviations and acronyms, instead use short synonyms, for instance: for "Cesarean section" instead of "CS" use "section" or "hysterectomy."

Abbreviations that may be used in the text without definition. Note, do not use periods in any abbreviation (ACTH not A.C.T.H.).

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ANOVA
ADP
ATP
BSA
cAMP
CL

DEAE-cellulose
DMSO
DNA
eCG
EDTA
EGF
ELISA
FSH
GH
GnRH
hCG
HEPE
ShMG
IVC
IVF
IVM
LH
MOET
MSH
mRNA
NAD
NADH
PBS
PGF2
PGFM
PIPES
PRID
PRL
RIA
RNA
SDS-PAGE
TRH
TRIS
tRNA
TSH

Units of Measure:
cpm - counts per min
dpm - disintegrations per min
g - gram
ga - gauge of hypodermic needle
h - hour
kg - kilogram
L - liter
mL - milliliter

vL - microliter
m - meter
min - minute
sec - second
v:v - volume ratio
wk - week
wt/vol - weight per volume
yr - year

Routes of treatment:
id - intradermal
im - intramuscular
iu - intrauterine
iv - intravenous
sc - subcutaneous
po - oral

Statistical expressions:
CV - coefficient of variation
df - degrees of freedom
F - variance ratio
NS - not significant
P - probability
SD - standard deviation
SEM - standard error of the mean
r - correlation coefficient

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ATA DA DEFESA DA DISSERTAÇÃO

Ata da defesa de dissertação da mestranda **Bianka Santana dos Santos,** realizada em 28 de fevereiro de 2005, como requisito final para obtenção do título de Mestre em Bioquímica.

Às 14:10 minutos do dia 28 de fevereiro de 2005, foi realizada, no Auditório Prof. Marcionilo Lins, a defesa de dissertação de **Bianaka Santana dos Santos,** aluna do Curso de Mestrado em Bioquímica/UFPE. Iniciando, a Profa. Dra.Vera Lúcia de Menezes Lima, Coordenadora do curso supra citado, fez a apresentação da aluna, de sua orientadora, ela própria, e da Banca Examinadora composta pelos professores doutores: Vera Lúcia de Menezes Lima, na qualidade de Presidente, Maria Tereza dos Santos Correia, Luana Cassandra Breitenbach Barroso Coelho, ás três do Depto. de Bioquímica/UFPE, e Edileine Dellalibera, do Núcleo de Ciências da Saúde/Faculdade Maurício de Nassau. Após as apresentações, a Sra. Presidente convidou a aluna para a apresentação de sua dissertação intitulada: **"Efeito de Dieta Suplementada com Óleo de Girassol sobre o Perfil Lipídico Plasmático e Seminal de Caprinos",** e informou, que de acordo com o Regimento Interno do Curso, o candidato dispõe de até 50 (cinqüenta) minutos para apresentação do trabalho e o tempo de argüição para cada examinador, juntamente com o tempo gasto pelo aluno para responder às perguntas será de 30 (trinta) minutos. A aluna procedeu a sua apresentação em 50 (cinqüenta minutos). Após a defesa, a Sra. Presidente convidou os membros da Banca Examinadora para ocupar seus lugares, passando a palavra para a Profa. Dra. Edileine Dellalibera, em seguida para Profa. Dra. Maria Tereza dos Santos Correia, e finalmente para a Profa. Dra. Luana Cassandra Breitenbach Barroso Coelho. Concluídas suas argüições, agradeceram e cumprimentaram a mestranda e sua orientadora. Com a palavra a Sra. Presidente, na qualidade de orientadora da aluna, fez alguns comentários e ,em seguida, a sessão foi suspensa para o julgamento pela Banca Examinadora, que se reuniu na Secretaria do Curso, na presença da Coordenadora. Após alguns comentários, a Banca decidiu, por unanimidade, conceder a menção **"Aprovada com Distinção"**. Nada mais havendo a tratar, lavrei a presente ata que vai assinada por mim, Secretário, e demais membros da Banca Examinadora. Recife, 28 de fevereiro de 2005.

A PRESENTE CÓPIA CONFERE
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RECIFE, 11 / 04 / 2005

Djalma Gomes da Silva
Assessor da Coordenação do Curso de
Mestrado em Bioquímica / CCB / UFPE