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مثبطات النمو في المصادر العلفية النباتية و أثرها على الأسماك: استعراض مرجعي

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الملخص:

إن إستخدام المصادر العلفية النباتية والتي تشمل البذور الزيتية وبذور البقوليات والحبوب والنباتات الدرنية كمساحيق في أعلاف الأسماك يعتبر محدودا ، وذلك راجع إلى وجود عدد من مثبطات النمو في هذه المصادر. ولعل من أهم هذه المثبطات، مثبطات الأنزيمات البروتينية ومركبات اللكتين ومركبات التان والجوسيبولات والفيتينات والجلوكوسينوليتات والصابونينات والسيانوجينات والمركبات الشبه قلوية والمركبات القليلة التسكر والمركبات العديدة التسكر الغير نشوية. لقد تم في هذه المراجعة التعريف بكل مثبط من المثبطات الهامة مع إستعراض الآثار التي تحدثها هذه المثبطات على الأسماك من خلال الحدود الدنيا و الحدود العليا لكل مثبط. لقد تم تقسيم الدراسات في هذه المراجعة إلى قسمين، دراسات تطرقت إلى أثر المثبطات على الأسماك من خلال إضافة هذه المثبطات بشكلها النقي إلى أعلاف الأسماك، بينما تطرق القسم الأخر من الدراسات إلى المثبطات التي تدخل في تركيب العلف من خلال إضافة المصادر النباتية المعروف باحتوائها على بعض المثبطات إلى الأعلاف بمستويات مختلفة. وأياً كانت الطريقة التي تمت بها دراسة أثر هذه المثبطات على الأسماك، فإن الآثار السلبية المترتبة على إضافتها قد تنوعت ما بين انخفاض في استساغة الأعلاف و انخفاض في الكفاءة الغذائية للأعلاف و انخفاض في معدلات الهضم للمغذيات بشكل عام، إضافة إلى الانخفاض في نمو الأسماك كنتيجة نهائية لهذه الآثار. كما تم استعراض الطرق المتبعة في تقليل الآثار السلبية لهذه المثبطات والتي تشمل المعالجة الحرارية، سواء

بالبخار الساخن المصاحب للضغط أو بالتسخين على الجاف، والمعالجة بالاستخلاص، سواء بالمذيبات أو بالماء الساخن من خلال التنقيع، وبإضافة الإنزيمات الهاضمة أو العوامل المؤكسدة.

الكلمات المفتاحية: مثبطات النمو، الأسماك، الأعلاف، المصادر العلفية النباتية.

المقدمة:

تعتبر مساحيق المصادر العلفية النباتية مصدرا رديفا لمساحيق الأسماك في تكوين الأعلاف السمكية بشكل عام، حيث تتميز هذه المساحيق النباتية بعدة مميزات جعل من إضافتها إلى أعلاف الأسماك أمرا حتميا في ظل الظروف العالمية الصعبة التي تواجه صناعة المساحيق السمكية والتي تشمل قلة المصادر وارتفاع التكلفة.

ولعل أهم ما يميز المساحيق النباتية عن غيرها من المساحيق الحيوانية بشكل عام والمساحيق السمكية بوجه خاص، هو أنها تتواجد بكميات كبيرة جدا تفوق كميات المساحيق الحيوانية والسمكية مجتمعة أضعاف مضاعفة، كما أنها تتوفر بأنواع متعددة ناهيك عن انخفاض أثمانها مقارنة بأثمان المساحيق السمكية. يضاف إلى ذلك احتواء الكثير منها على نسب مرتفعة من البروتينات.

إن استخدام مساحيق من المصادر العلفية النباتية في أعلاف الأسماك معروف منذ عدة عقود، حيث أظهرت نتائج الدراسات في مجال تغذية الأسماك نجاحا نسبيا ملحوظا في هذا المجال خصوصا في حال الاستعاضة الجزئية عن مساحيق الأسماك بالمساحيق النباتية، إلا أن تلك الدراسات لم يحالفها الحظ بعد فيما يتعلق بالاستعاضة الكلية. وقد أرجع السبب في ذلك إلى عدة عوامل لعل من أهمها هو

وجود مثبطات للنمو في هذه المساحيق (Liener, 1980; Huisman *et al*., 1989; Krogdahl, 1989; NRC, 1993).

تعرف مثبطات النمو، بأنها تلك المواد أو المواد الناتجة عنها داخل جسم الكائن التي تصطدم مع مكونات الأعلاف وتحدث أثرا سلبيا على كفاءة وإنتاجية وصحة الكائن. وقد قسمت هذه المثبطات إلى عدة مجموعات وذلك بناء على عنصرين، الأول هو تركيبها الكيميائي الذي تتصف به والثاني هو الآثار السلبية التي تحدثها هذه المثبطات في حال تناولها من قبل الأسماك. ومن خلال هذا التقسيم تم حصر المثبطات في أربعة مجاميع، فهناك المثبطات التي تؤثر على هضم وإستخدامات البروتينات والتي تشمل مثبطات الأنزيمات البروتينية Protease inhibitors واللكتينات Lectins ومركبات التان Tannins، ومجموعة المثبطات التي تؤثر على إمتصاص وإستخدامات المعادن وتشمل الجوسيبولات Gossypols والفيتينات Phytates، ومجموعة مثبطات الجلوكوسيدات Glucosides وتشمل الجلوكوسينوليتات Gulocosinolates والصابونينات Saponins والسيانوجينات Cyanogens، أما المجموعة الرابعة فتشمل ما تبقى من هذه المثبطات ولعل من أهمها أو ما سيتم التطرق إليه في هذا الإستعراض، هي المركبات الشبه القلوية Alkaloids والمركبات القليلة التسكر والمركبات العديدة التسكر الغير نشوية Oligosaccharides and Non Starch Polysaccharides.

كما سيتم في هذا الاستعراض المرجعي ذكر مثبطات النمو الرئيسية التي تتواجد في المصادر النباتية والتي من المتوقع أن تحل محل المساحيق السمكية بصورة كلية، حيث سيتم تسليط الضوء على الآثار السلبية الناتجة من إضافة هذه المثبطات إلى أعلاف الأسماك المختلفة سواء بصورتها النقية المستخلصة من المصدر النباتي أو بصورتها الطبيعية التي تتواجد عليها في ذلك المصدر، كما سيتم تسليط الضوء على

الطرق المتبعة في كيفية التخلص من هذه المثبطات أو التقليل من تراكيزها في المصادر النباتية.

مثبطات الأنزيمات البروتينية Protease inhibitors :

تعتبر مثبطات الإنزيمات البروتينية واسعة الإنتشار في كثير من المصادر النباتية التي تدخل في تركيب أعلاف الأسماك، ومنها على وجه الخصوص البقوليات و البذور الزيتية (Norton, 1991). وتعود أهمية هذه المثبطات إلى وجودها في مصادر علفية نباتية هامة إضافة إلى قوة تأثيرها على بعض الإنزيمات. و توجد مجموعتان من مثبطات الإنزيمات البروتينية، تعرف الأولى بإسم مجموعة كونيتز Kunitz وهي تستهدف في تأثيرها إنزيم التربسين trypsin inhibitor وتتصف هذه المجموعة بأنها حساسة للحرارة والأحماض، أما المجموعة الثانية فإنها تستهدف كل من أنزيم التربسين وأنزيم الكيموتربسين وتعرف بإسم مجموعة بومان- بيرك Bowman-Birk وتتصف هذه المجموعة بأنها مقاومة للحرارة (Norton, 1991).

تتواجد مثبطات أنزيم التربسين في منتجات فول الصويا التجارية بمستوى يتراوح ما بين ٢- ٦ مجم/جم (Synder and Kwon, 1987)، كما أنها تتواجد في أنواع عديدة من المملكة النباتية حيث تتواجد في معظم بذور البقوليات والحبوب والبذور الزيتية. وتختلف الأسماك فيما بينها في قدرتها على تحمل أثر مثبطات إنزيم التربسين، حيث وجد أن أسماك البلطي *Oreochromis mossabicus* (Jackson *et al*., 1982) وأسماك المبروك *Cyprinus carpio* (Makkar and Becker, 1999) وأسماك التراوت القزحية *Oncorhynchus mykiss* (Rumsey *et al*., 1993) وأسماك القراميط الأمريكية *Ictalurus punctatus* (Wilson and Poe, 1985) وأسماك السلمون *Salmo salar* (Olli *et al*., 1994a) وأسماك الشعم البحري ذو الرأس

اللامع *Sparus aurata* (Robaina *et al*., 1995) عند تناولها أعلافا تحتوي على مصادر نباتية يعرف عنها إحتوائها على نسب من مثبطات أنزيم التربسين مثل مسحوق فول الصويا ومسحوق بذور الترمس ومسحوق بذور اللفت، أنها تبدي مستويات من النمو متقاربة مع المستويات التي تبديها الأسماك التي تناولت الأعلاف القياسية (مسحوق السمك) والتي تخلو من هذه المثبطات. كما لوحظ أن مستويات من مثبطات أنزيم التربسين تقدر بـ ١,٦ مجم/جم أو أكثر قد أعاقت نمو أسماك البلطي النيلي *Oreochromis niloticus*، إلا أن هذه الأسماك قد قاومت ونمت نموا جيدا عندما إنخفض مستوى مثبطات الإنزيم إلى ٠,٦ مجم/جم (Wee and Shu, 1989). في بعض الأسماك مثل أسماك المبروك *Cyprinus carpio* لم تلاحظ فروقات في النمو عندما تناولت نوعين من الأعلاف معالجة حراريا يحتوي الأول على ٢٤,٨ مجم/جم بينما يحتوي الأخر على مستويات تتراوح ما بين ١,٣- ٨,٣ مجم/جم من مثبطات أنزيم التربسين، مما يعطي إنطباعا بأن لهذه الأسماك القدرة على تحمل مدى واسع من هذه المثبطات (Makkar and Becker, 1999). كما وجد أن أفضل نمو تبديه إصبعيات أسماك القراميط الأمريكية *Ictalurus punctatus* عندما يكون مستوى مثبط أنزيم التربسين في العليقة عند المستوى ٢,٢ مجم/جم (Wilson and Poe, 1985).

من جانب أخر، فقد وجد أن أسماك التراوت القزحية *Oncorhynchus mykiss* حساسة جدا لمثبطات الأنزيمات البروتينية الموجودة في فول الصويا، وأن هناك علاقة مباشرة بين كمية مثبطات أنزيم التربسين في العليقة وتوفر كل من البروتين والطاقة (Sandholm *et al*., 1976; Krogdahl *et al*., 1994). ولقد توقع Dabrowski *et al.* (1989) أن ضعف إفراز أنزيم الكيموتربسين chymotrypsin في أسماك التراوت

القزحية *Oncorhynchus mykiss* التي تناولت أعلافا تحتوي على فول الصويا، هو نتيجة لتثبيط أو توقف الآلية التي يتم بها إفراز الإنزيم من البنكرياس.

لقد وجد أن أعلى مستوى يتم فيه إنتاج أنزيم التربسين في أسماك السلمون الأطلسي *Salmo salar* عندما يكون مستوى مثبط أنزيم التربسين في العليقة عند ٤,٨ مجم/جم (Olli *et al*., 1994a). كما لوحظ أن أسماك التراوت القزحية *Oncorhynchus mykiss* كانت قادرة على التعويض الجزئي لأثر مثبط أنزيم التربسين بزيادة الإفراز الإنزيمي و بتعزيز إمتصاص البروتين في الجزء الأخير من الأمعاء (Krogdahl *et al*., 1994). ومما سبق ذكره، يبدو أن أغلب الأسماك المستزرعة قادرة على تعويض أثر مثبطات أنزيم التربسين عليها عندما يتواجد في الأعلاف بمستوى أقل من ٥ مجم/جم عن طريق زيادة إفراز أنزيم التربسين.

لقد لوحظ أنه من الممكن تقليل المستويات الحرجة من مثبطات أنزيم التربسين الموجودة في المصادر العلفية النباتية إلى مستويات مأمونة عن طريق المعالجة الحرارية بالبخار الساخن (autoclaving) لمدة تتراوح ما بين ١٥- ٣٠ دقيقة (Norton, 1991). ولكن يجب توخي الحذر عند استخدام هذه الطريقة حيث أن أية زيادة في فترة التسخين قد تؤثر على مكونات المصدر العلفي بفقد بعض الأحماض الأمينية الأساسية خصوصا اللايسين (NRC, 1993) مما يتسبب في إنخفاض القيمة الغذائية لهذا المصدر.

مركبات اللكتين Lectins :

تعرف مركبات اللكتين بإسم phytohaemagglutinins و هي تتواجد في كثير من بذور البقوليات ولديها القدرة على ربط أجزاء من مركبات الهيدروكربونات المتواجدة في أغشية الخلية. وتعتبر اللكتينات بروتينات مقاومة جزئيا للتحلل

بالأنزيمات الهاضمة في الأمعاء، حيث تشمل أثارها البيولوجية الإضرار بعمليات الأيض وتغير الشكل الخارجي لخملات الأمعاء الدقيقة (Grant, 1991).

تعود سمية لكتين فول الصويا الغير منزوع الدهن إلى قدرة هذه المركبات على الإرتباط بشدة مع خملات السطح الخارجي لخلايا الجزء الأخير من الأمعاء الدقيقة في أسماك السلمون الأطلسي *Salmo salar* (Hendricks *et al.,* 1990). لقد شاهد Van der Ingh *et al.* (1991) اختلافات واضحة على الطبقة المخاطية لمنطقة الأمعاء الدقيقة (الجزء الأخير) في أسماك السلمون الأطلسي نتيجة لتناولها مسحوق فول الصويا الكامل الدهن، حيث لوحظ زيادة عددية للخلايا الكأسية في الطبقة الطلائية للأمعاء و نقص حاد في التجاويف الخلوية مع تكون حويصلات ما بين الخملات الدقيقة للخلايا، و لقد فسرت هذه التغيرات على أنها نتيجة للإفراز المفرط لمادة المخاط من خلايا الأمعاء الدقيقة كسبب للإلتهابات الشديدة التي أحدثتها مركبات اللكتين. وعلى هذا فمن المعتقد أن الإلتهابات التي تحدثها مركبات اللكتين لخلايا الأمعاء والتي تتسبب في الإفراز المفرط لمادة المخاط قد تضعف من النشاط الإنزيمي و كفاءة الإمتصاص في الأمعاء.

في دراسات أخرى، ثبت أن نمو أسماك المبروك الشائع *Cyprinus carpio* لم يتأثر نتيجة لتغذيتها على مستويات سواء منخفضة (١,٢ وحدة haemagglutination) أو عالية (٥١ وحدة haemagglutination) من اللكتين (Makkar and Becker, 1999). كما أن الأعراض الأخرى و المرتبطة بتأثير اللكتين مثل تلف العضلات وإستنزاف الدهون من الأنسجة الدهنية و تضخم الكبد لم تظهر على هذه الأسماك (Grant, 1991).

يمكن التخلص من مركبات اللكتبن عن طريق المعالجة بالماء الساخن، أو عن طريق إستخدام الأوتوكليف (Grant, 1991). كما قام Aregheore *et al*. (1998) بتخفيض مستوى اللكتين في مسحوق بذور Jatropha من ١٠٢ إلى ١,١٧ وحدة عن طريق التسخين بالبخار تحت درجة حرارة ١٠٠ درجة مئوية ولمدة ١٠ دقائق.

مركبات التان Tannins :

تعتبر مركبات التان مركبات ثانوية لعدة مركبات كيميائية مختلفة واسعة الإنتشار في المملكة النباتية وهي تنقسم إلى مجموعتين: مجموعة مركبات التان القابلة للتحلل hydrolysable ومجموعة مركبات التان الكثيفة condensed.

ولهذه المركبات تأثيرات سلبية عند وجودها في عليقة الأسماك ومنها تعارضها مع عمليات الهضم سواء بربط الإنزيمات الهاضمة أو بربط مكونات العليقة مثل البروتينات والمعادن، بالإضافة إلى ذلك فإن لمركبات التان القدرة على تقليل إمتصاص فيتامين B12 (Liener, 1989). في دراسات على أسماك المبروك الشائع *Cyprinus carpio* ثبت أن هذه الأسماك لها القدرة على مقاومة تأثير تركيز ٢٪ من مسحوق التان من نوع Quebracho، وهو من النوع الكثيف، من دون ظهور أي أثر سلبي على النمو، بينما أظهرت النسبة ذاتها من نوع حمض التان tannic acid، وهو من النوع القابل للتحلل، تأثيرا مخالف أدى إلى عدم قبول الأعلاف بعد أربعة أسابيع من بداية الدراسة (Becker and Makkar, 1999)، وهذه النتيجة تتعارض مع ما ذكره Al-Owafeir (1999) الذي وجد أن إضافة ١٪ سواء من مسحوق حمض التان النقي أو مسحوق catechin (إحدى مركبات التان الكثيفة) إلى أعلاف أسماك البلطي النيلي *Oreochromis niloticus* أو أسماك القراميط الإفريقية *Clarias gariepinus* لم تحدث فروق معنوية فيما يخص قبول الأعلاف ومعامل هضم البروتين

ومستويات النمو عند مقارنتها بالأسماك التي تغذت على أعلاف خالية من تلك المركبات.

بخلاف مركبات التان الكثيفة، فإن المركبات القابلة للتحلل من السهل تفككها داخل الأجهزة الحيوية للأسماك مكونة مركبات صغيرة لها القدرة على الدخول إلى مجرى الدم، ومع مرور الوقت تسبب التسمم لبعض الأعضاء مثل الكبد والكلى. لقد أوضحت بعض الدراسات أن مركبات التان الكثيفة الموجودة في لب جوز الهند المجفف copra بنسبة ٢,٤٪ ممكن أن تكون سببا في إنخفاض نمو إصبعيات أسماك البلطي *Oreochromis mossabicus* وأسماك الروهو *Labeo rohita* عند إضافتها بنسبة ٢٠ أو ٢٥٪ (Jackson *et al*, 1982; Mukhopadhyay and Ray, 1999a). أعلاف أخرى تحتوي على مركبات التان الكثيفة مثل مسحوق بذور اللفت ومسحوق بذور البازلاء قد ثبتت مقاومة بعض الأسماك لها من المستويات المتوسطة وحتى المستويات المرتفعة. وفي دراسة مخبرية (*in vitro*)، وجد أن مسحوق الفول *Vicia faba* والذي يحتوي على مركبات التان الكثيفة قد خفض معامل هضم البروتين أكثر مما خفضه مسحوق فول الصويا (Grabner and Hofer, 1985). ولقد وجد أن الإختلافات كانت أكثر وضوحا عندما تم تمثيل معدة سمك المبروك الشائع *Cyprinus carpio* أكثر مما تم تمثيله لمعدة أسماك التراوت القزحي *Oncorhynchus mykiss*. وهذه الإختلافات توضح الفروقات في تحمل الأسماك المختلفة لمركبات التان في الأعلاف.

يعرف عن مركبات التان تفاعلها مع مثبطات نمو أخرى، على سبيل المثال تفاعل مركبات التان مع مركبات اللكتن lectins والذي يعمل على تقليل أثر التان السلبي على أنزيم الأميليز (Fish and Thompson, 1991). والتفاعل ما بين مركبات

التان ومركبات الجلوكوسيدات السيانوجينية cyanogenic glycosides والذي يقلل من الأثر السلبي للأخير (Goldstein and Spencer, 1985).

توجد عدة طرق للتخلص من مركبات التان في مصادر العليقة أو التقليل من أثرها، فمن الطرق الموصى بها للتخلص من مركبات التان نزع قشور البذور، حيث يعرف أن هذه القشور غنية بمركبات التان. كما أن المعالجة الحرارية بجهاز الأوتوكليف أو المعالجة القلوية تقلل من نسبة التان في المصادر العلفية (Griffiths, 1991). كما تم ملاحظة إنخفاض مستوى التان في مسحوق السمسم من ٢٠- ١٠ جم/كجم بعد معالجته بالتخمر مع بكتيريا حمض اللكتك (Mukhopadhyay and Ray, 1999b). كما أن المعالجة بالعوامل المؤكسدة وإضافة مواد رابطة مثل جليكولات الاثيلين المتعددة polyethylene glycol إلى الأعلاف المحتوية على مركبات التان قد يقلل من الأثر السلبي لها على الأسماك (Makkar *et al*., 1995a; Makkar and Becker, 1996).

الجوسيبولات Gossypols :

تعتبر الجوسيبولات مركبات فينولية متعددة تتواجد في الغدد الصبغية للنباتات القطنية من جنس *Gossypium* وفي أجناس أخرى من فصيلة Malvaceae. تتسبب الجوسيبولات في حالة وجودها في العليقة بإنخفاض في النمو وتغيرات غير سوية في الأمعاء وغيرها من الأعضاء الداخلية للأسماك (Berardi and Goldblatt, 1980). يعتبر إتحاد البروتين مع الجوسيبول الموجود في الأعلاف و تكون مركب معقد، السبب الرئيس لنقص بعض الأحماض الأمينية مثل حمض الميثايونين methionine والذي يعتبر الحمض الأساس لعمليات أيض الدهون (Herman, 1970)، حيث وجد إرتفاع في تركيز بعض الأحماض الدهنية مثل palmitic acid و linoleic acid

وإنخفاض في تركيز Oleic acid في عضلات أسماك البلطي النيلي *Oreochromis niloticus* عندما تناولت أعلافا تحتوي على مسحوق بذور القطن الغير منزوع الدهن (Ofojekwu and Ejike, 1984).

في دراسة على أصبعيات أسماك التراوت *Oncorhynchus mykiss* وجد أن نمو هذه الأسماك قد إنخفض بمقدار النصف مقارنة بالمجموعة الشاهدة عندما تغذت على عليقة تحتوي على ١٪ خلات الجوسيبول gossypol acetate (Roehm *et al*., 1967)، بينما تسببت نسبة ٢٪ في رفض للعليقة من قبل الأسماك. حيث إرتبطت كمية كبيرة من الجوسيبول بأنسجة الكبد والطحال و الكلى، ولقد ظلت هذه الكمية حتى بعد أن تغذت الأسماك على أعلاف خالية من الجوسيبول لمدة ١٠ أسابيع. كما تأثر نمو أسماك البلطي الأزرق *Oreochromis aureus* عندما تغذت على جوسيبول حر بنسبة ٠,٠١٢٪ (Robinson *et al*., 1984). من جانب آخر فقد وجد أن إضافة مسحوق بذور القطن بمستويات عالية إلى أعلاف الأسماك قد أعطى نتائج مقاربة للأعلاف القياسية (مسحوق سمك) من حيث النمو لأسماك القراميط الأمريكية *Ictalurus punctatus* (Reigh, 1999) وأسماك البلطي الموزمبيقي *Oreochromis mossambicus* (Jackson *et al*., 1982).

يتسبب مسحوق بذور القطن الخام (٠,٠٢٩٪ جوسيبول حر) في حالة إستخدامه في الأعلاف بإنخفاض في النمو وتغيرات مرضية في أنسجة الأمعاء في أسماك التراوت القزحية *Oncorhynchus mykiss* (Herman, 1970)، وموت لخلايا الكبد وزيادة في سماكة الشعيرات الدموية في الكلى وتراكم لحبيبات صبغيات السيروید ceroid في الكبد. كما ثبت أن ٠,١٪ من الجوسيبول قد تسبب في تحلل سريع وشديد للخلايا الدهنية الكبدية وتهتك واسع لخلايا الكبد.

يعرف أن مركبات الجوسيبول تتسبب في إحداث مشاكل مرضية للجهاز التناسلي في الثديات من خلال التأثير المباشر على الخلايا التناسلية أو على الغدة النخامية أو على إفراز الغدد الهرمونية (Randel *et al.*, 1992). كما ثبت أن مسحوق بذور القطن بنسبة ٨٪ من وزن العليقة قد تسبب في التغير في نشاط الخلايا المنوية وزيادة غير طبيعية في أعدادها و تغير في أنسجة الخصي (Salaro *et al.*, 2000). وفي دراسة مخبرية (*in vitro*)، إتضح أن ٢٠٠ ميكرومول من الجوسيبول قد تسببت في إيقاف حركة الحيامن في سمكة الفرخ الأصفر *Perca flavescens* (Ciereszko and Dabrowski, 2000).

تعتبر عملية إنتخاب أصناف جديدة من بذور القطن من الطرق الفعالة والشائعة في تقليل محتوى الجوسيبول في بذورالقطن، حيث تنتج أصنافا من البذور الخالية من الغدد التي تحتوى على النسبة الكبيرة من الجوسيبول الموجودة في البذور (Robinson *et al.*, 1984). كما أن عملية إستخلاص الدهن بالمذيبات أو عمليات الكبس للبذور من العمليات التي تقلل من محتوى الجوسيبول في بذور القطن (Francis *et al.*, 2001).

الفيتينات Phytates :

تتواجد الفيتينات بشكل واسع في بذور النباتات حيث تكون لها القدرة على ربط أيونات المعادن الثنائية والثلاثية مثل أيونات الكالسيوم والمغنسيوم والخارصين والنحاس والحديد فتكون هذه الأيونات غير متوفرة عند التغذي عليها (Duffus and Duffus, 1991). ويعرف عن الفيتينات أنها غير قابلة للتحطم أو التكسير بواسطة أنزيمات الحيوانات الغير مجترة، ولهذا فإن تواجدها في الأعلاف يقلل من توفر عنصر الفسفور حيويا لتلك الحيوانات (Liener, 1989). كما يعرف عن الفيتينات أنها

تكون مركبات معقدة عند ارتباطها بالبروتينات فتكون غير متوفرة أيضا للحيوانات (Richardson *et al.*, 1985).

وتتواجد الفيتينات في النباتات التي تستخدم كمصدر لأعلاف الأسماك مثل مسحوق فول الصويا ومسحوق بذور اللفت ومسحوق بذور السمسم حيث تحتوي على ما نسبته ١٠- ١٥ و ٥٠- ٧٥ و ٢٤ جم/كجم فايتين على التوالي. ويتأثر النمو سلبا في كثير من الأسماك المستزرعة الشائعة مثل أسماك المبروك *Cyprinus carpio* وأسماك البلطي *Oreochromis niloticus* وأسماك التراوت *Oncorhynchus mykiss* وأسماك السلمون *Salmo Salar* عند تناولها أعلافا تحتوي على الفيتينات. ولقد تم التأكد من الأثر السلبي للفيتين على الأسماك من خلال إضافة هذا المركب صناعيا إلى أعلافها. حيث تبين أن أسماك التراوت *Oncorhynchus mykiss* قد إنخفض نموها عندما أضيف إلى أعلافها حمض الفيتين phytic acid بمقدار ٥ جم/كجم (Spinelli *et al.*, 1983). حيث وجد أن تكون المركب المعقد ما بين الفيتين والبروتين هو السبب الرئيس لانخفاض النمو كنتيجة لعدم هضم هذا المركب. ولقد تم تسجيل نفس النتيجة عندما أضيف حمض الفيتين بمقدار ٢٥,٨ جم/كجم إلى أعلاف أسماك السلمون من نوع الشينوك *Oncorhynchus tshawytscha* (Richardson *et al.*, 1985). حيث شوهد إنخفاضا في معامل التحول الغذائي وإنخفاضا في مستوى بقاء البروتين في أنسجة تلك الأسماك. ولقد أرجع السبب إلى ربط حمض الفيتين، المضاف بكميات كبيرة، بعنصر الخارصين وجعله غير متوفرا حيويا، حيث لوحظ، في نفس الدراسة، تحسنا في العوامل المذكورة عند إضافة كمية إضافية من عنصر الخارصين إلى الأعلاف. كما تم مشاهدة نموا غير طبيعيا في شكل خلايا كل من الكلى والقناة الهضمية والغدة الدرقية، كما تكونت إنتفاخات في خلايا الزوائد الأعورية pyloric caecae ولقد كانت جميع هذه

الشواهد استنتاجاً للأثر السلبي لحمض الفيتين. كما تم مشاهدة حدوث عتمة في عيون أسماك السلمون اليافعة عندما تغذت على أعلاف تحتوي على نسبة مرتفعة من الفيتين مع نسبة منخفضة من الخارصين مما يوضح تكون مركب معقد ما بين الفيتين والخارصين (Richardson *et al*., 1985).

في دراسة على أسماك المبروك *Cyprinus carpio*، تبين أن حمض الفيتين (٥ و ١٠ جم/كجم) قد أحدث إنخفاضا في نمو الأسماك و تضخما في خلايا الطبقة الطلائية للقناة الهضمية (Hossain and Jauncey, 1993). كما تم مشاهدة إنخفاضا في تركيز عنصر الخارصين في عظام أسماك التراوت *Oncorhynchus mykiss* اليافعة عندما تناولت أعلافا تحتوي على بروتين من مسحوق بذور اللفت الغير معالج، حيث إحتوى هذا المسحوق على ٥٣- ٧٥ جم/كجم من حمض الفيتين (Teskeredzic *et al*., 1995).

لقد أتضح من دراسة سابقة، أن إضافة إنزيم الفيتيز phytase تعادل الأثر السلبي لحمض الفيتين، وأن توفر عنصر الفسفور حيويا في المصادر النباتية يرتفع من ٩,٧٪ و ٤٨,٤٪ إلى ٤٦,٢٪ و ٧٥,٦٪، على التوالي بإضافة إنزيم الفيتيز إلى هذه المصادر (Riche and Brown, 1996). كما إتضح من خلال الدراسات أن تحضين مسحوق الصويا مع إنزيم الفيتيز قد حسن من استخدامات البروتين ومعامل هضمه، كما حسن من مستويات تواجد العناصر المعدنية (الكالسيوم، المغنسيوم، الخارصين، والفسفور) في أنسجة أسماك السلمون الأطلسي *Salmo salar* عندما أضيف هذا المسحوق إلى أعلافها (Storebakken *et al*., 1998; Vielma *et al*., 1998).

تتركز الفيتينات و بالأخص في الحبوب في منطقة السويداء الخارجية outer endosperm، لذلك فإن طحن هذه الحبوب يعمل على التقليل من محتواها من الفيتين بدرجة كبيرة. كما أن عملية التخمير fermentation تقلل من محتوى الفيتين في الحبوب كنتيجة لإفراز أنزيم الفيتيز من الخمائر yeasts أو من بكتيريا حمض اللكتيك (Duffus and Duffus, 1991). كما وجد أن المعالجة الحرارية (autoclaving) تقلل من الفيتين في مسحوق السمسم ومسحوق الكتان حتى ٧٤٪ و٧٢٪ على التوالي (Hossain and Jauncey, 1990).

الجلوكوسينوليتات Gulcosinolates :

الجلوكوسينوليتات عبارة عن مركبات تحمل عنصر الكبريت و تعرف بالجلوكوسيدات الكبريتية thioglucosides. والجلوكوسيدات، هي المركبات التي تعطي عند هدرجتها مركبين، الأول سكري والأخر مركب غير سكري. وتتواجد هذه المركبات في العائلة الصليبية (ذوات الفلقتين) Cruciferae وهي مسؤولة عن الطعم الحاذق في هذه النباتات مثل نبات الخردل واللفت والجرجير وغيرها من النباتات التي تنتمي إلى هذه الفصيلة. وتتواجد هذه المركبات متلازمة مع أنزيمات الثايوجلوكوسايديز thioglucosidase في النبات، إلا أنهما يكونان في أماكن مختلفة من الخلية. وفي حال إندماج هذه المركبات مع تلك الإنزيمات نتيجة لتحطم الخلية فإن الأثر السلبي لهذه المركبات يكون واضحاً (Duncan, 1991).

تعتبر الجلوكوسينوليتات من العوامل المثبطة الرئيسية في مسحوق بذور اللفت rapeseed (*Brassica spp.*) ورقائق بذور الخردل (Duncan, 1991) و يعتبر هذين النباتين مصدرين هامين من مصادر أعلاف الأسماك. ولقد حسن علماء الوراثة النباتية من نوعية كل من بذور *Brassica napus* وبذور *B. campestris* وذلك

بتخفيض مستوى الجلوكوسينوليتات فيها إلى أقل من ٣ مجم/جم حيث عرفت هذه البذور لاحقا بإسم الكانولا canola. ولقد وجد Higgs *et al.* (1982) أن مسحوق بذور اللفت (١٦٪) قد أثر على تركيب الغدة الدرقية في أصبعيات أسماك السلمون من نوع الشينوك *Oncorhynchus tshawytscha* بينما لم يؤثر مسحوق الكانولا أو يحدث أي أثر سلبي، إلا أن النمو كان متقاربا مع العينة الشاهدة في كلا الحالتين. ولقد إتصفت الغدد الدرقية المصابة بوجود إنقسامات خلوية غير سوية واضحة وظهور تضخم مسامي Follicular hypertrophy، مما تعتبر مؤشرات لنشاط غير طبيعي لهذه الغدد. كما أن الخلايا الطلائية بدت أطول في الأسماك المصابة من نظيرتها في الأسماك السليمة وإحتوت على مواد غروية، كما لوحظ أن هناك إنقسامات ميتوزية عديدة، وهي في مجملها أعراض واضحة لمحاولات الأسماك في المحافظة على مستوى هرمون الغدة الدرقية في الدم من خلال زيادة نشاط الغدة الدرقية. كما تم مشاهدة نفس الأثر على أسماك المبروك العادي *Cyprinus carpio* عندما تغذت على أعلاف تحتوي على ٣,٣ جم/كجم من الجلوكوسينوليت النقي (Hossain and Jauncey, 1989)، وكذلك على أسماك البلطي الموزمبيقي *Oreochromis mossambicus* عندما تغذت على أعلاف تحتوي على ٢,٥ جم/كجم جلوكوسينوليت (Davies *et al.*, 1990). أما أسماك التراوت *Oncorhynchus mykiss* وأسماك التربوت *Psetta maxima* فقد وجد أن لها القدرة على تعويض إنخفاض وظائف الغدة الدرقية الناتج عن وجود الجلوكوسينوليت في العليقة من خلال النشاط العالي لأنزيم الديودينيز deiodinase والذي يحول الدرقين thyroxine (هرمون الغدة الدرقية) إلى مركب نشط يعرف بإسم triiodothyronine (Burel *et al.*, 1998, 2000a). ولقد كان من المستغرب أن أسماك التربوت *Psetta maxima* التي تغذت على نسب عالية من الجلوكوسينوليت (١١,٦ ميكرومول/جم) في أعلاف

من مسحوق بذور اللفت كان تركيز الدرقين وتركيز triiodothyronine بها في المستويات الطبيعية، علما أنه تم ملاحظة نشاط عالي لإنزيم deiodinase في بلازما الدم (Burel *et al.*, 2000b). وقد يعود هذا إلى عدم تحطم الجلوكوسينوليت إلى منتجات ثانوية سامة في مسحوق بذور اللف الغير معالج.

إن هضم نسب بسيطة من الجلوكوسينوليت (١,٤ ميكرومول/جم) يؤدي إلى إنخفاض في النمو، وإنخفاض في كفاءة العلف في أسماك التراوت *Oncorhynchus mykiss*، إلا أن هذا الأثر لم يتفاقم عندما إرتفعت نسبة الجلوكوسينوليت إلى ١١,٦ ميكرومول/جم، لكن تم ملاحظة إنخفاض شديد في النمو عندما وصل مستوى الجلوكوسينوليت في العلف إلى ١٩,٣ ميكرومول/جم علما بأن نشاط الغدة الدرقية قد تأثر بالمستويات المنخفضة (Burel *et al.*, 2000c).

تعتبر المعالجة الحرارية عن طريق الطبخ بالضغط البخاري فعالة في تخفيض مستوى الجلوكوسينوليت في المصادر العلفية من ٤٠ إلى ٢٦ ميكرومول/جم (Burel *et al.*, 2000a)، كما تعتبر عملية التنقيع في الماء إحدى الطرق المنخفضة التكاليف والفعالة في تخفيض مستوى الجلوكوسينوليت (Makkar and Becker, 1997).

الصابونينات Saponins :

من المعروف تاريخيا، أن مادة الصابونين تستخدم كمادة سامة للأسماك. و يتواجد الصابونين في كثير من المصادر العلفية النباتية، ويتراوح تركيزه ما بين ١٨- ٤١ مجم/كجم في مختلف بذور البقوليات و٦٧ مجم/كجم في مسحوق فول الصويا المحمص المنزوع الدهن (Fenwick *et al.*, 1991). ويعتبر الصابونين ساما للأسماك وقاتلا عند وضعه في الماء حيث يعمل على تلف الخلايا الطلائية في الخياشيم، حيث تم مشاهدة نفوق لأسماك البلطي خلال ٥- ٦ ساعات من تأثير

مادة الصابونين الموجودة في بذور الشاي *Camellia sinensis* بتركيز ١٠٠ جزء في المليون (De *et al.*, 1987).

يتواجد الصابونين في مسحوق بذور الترمس lupin بنسبة ١,١٪ و في نبات alfalfa بنسب تتراوح ما بين ٠,٠٣- ١,٥٪، و قد يعزى إلى هذه النسب إنخفاض نمو أسماك التراوت *Oncorhynchus mykiss* (de la Higuera *et al.*, 1988) وأسماك البلطي *Oreochromis mossambicus* (Olvera-Novoa *et al.*, 1990) عندما تغذت على أعلاف تحتوي على هذه المصادر النباتية. إلا أن بعض الباحثين لم يلاحظوا أي أثر سلبي على نمو أسماك السلمون الأطلسي *Salmo salar* عندما تمت تغذيتها على أعلاف تحتوي على مسحوق فول الصويا به نسب من مادة الصابونين تتقارب مع النسب السابقة، إلا أن نفس الباحثين، إضافة إلى ذلك، قد وجدوا أن مستخلص مسحوق فول الصويا الكحولي قد أثر على نمو هذه الأسماك سلبا و أحدث إلتهابات في الجهاز الهضمي للسمكة وانخفاضاً في إنتاج المادة المخاطية في الأمعاء (Krogdahl *et al.*, 1995).

وفي دراسة أخرى (Bureau *et al.*, 1998)، تم ملاحظة أن أسماك التراوت *Oncorhynchus mykiss* أكثر تحملا من أسماك السلمون لتركيز مادة الصابونين المستخلص من مسحوق فول الصويا. حيث تبين أن أسماك السلمون من نوع *Oncorhynchus tshawytscha* قد تغير شكل خلايا جهازها الهضمي نتيجة لتغذيتها أعلافا تحتوي على مادة الصابونين، بينما لم يتبين هذا الأثر على خلايا الجهاز الهضمي لأسماك التروات، إلا أن هذين النوعين من الأسماك قد تأثرت أغشيتهما المخاطية تأثرا شديدا عندما إستهلكا أعلاف تحتوي على مادة الصابونين المستخلصة من أشجار الكويلاجا *Quillaja bark* بتركيز ١,٥ جم/كجم. كما

شوهد نفس الأثر على أسماك البلطي النيلي *Oreochromis niloticus* وأسماك القراميط الأفريقية *Clarias gariepinus* عندما تغذت على نفس المستخلص بتركيز ١٠ جم/كجم (Al-Owafeir, 1999).

إن الأثر السلبي للصابونين يعود إلى قدرة هذه المادة على إحداث توتر سطحي للأغشية الخلوية مما يتسبب في إحداث إلتهابات للخلايا. إلا أن هذه القدرة تختلف حسب المصدر الذي تم إستخلاص مادة الصابونين منه. فعلى سبيل المثال، وجد أن مادة الصابونين المستخلصة من فول الصويا أقل تأثيرا من مادة الصابونين المستخلصة من نبات *Gypsophylla*. وفيما يتعلق بقدرة هذه المادة على التأثير على معدل النفاذية من خلال الأغشية الخلوية، فبعض الصابونينات تعمل على زيادة معدل النفاذية في الخلايا المخاطية للأمعاء الدقيقة وبهذا فهي تعطل النقل الإيجابي للمغذيات (Johnson *et al.,* 1986). ومن خصائص الصابونينات، قدرتها على لعب دورا في تقليل معدلات النمو في الأسماك من خلال التأثير على معامل الهضم في البروتين (Shimoyamada *et al.*, 1998)، وقد يكون ذلك عن طريق إتحاد الصابونين مع إنزيم الكيموتربسين chymotrypsin ليتكون مركب كبير معقد يصعب تكسيره أو إمتصاصه (Potter *et al.*, 1993). كما أن تكون بعض المركبات المعقدة بين الصابونينات وبعض المثبطات الأخرى مثل التان tannin يعمل على تثبيط الأثر السلبي لكل منهما (Makkar *et al*., 1995b).

يعرف عن مركبات الصابونين أن لها القدرة العالية على الذوبان في الماء، عليه، فإن إستخلاص مادة الصابونين بالماء قد يزيل الجزء الأكبر من هذه المادة من المصادر العلفية الموجودة بها. و تعتبر هذه الطريقة فعالة و مأمونة بحيث أن بقية مكونات

المصادر العلفية الأخرى لن تتأثر سلبا بهذه الطريقة و لن تتأثر القيمة الغذائية لها أيضا (Price *et al*., 1987).

السيانوجينات Cyanogens :

السيانوجينات مركبات تتواجد بتركيزات عالية في عدد من الحبوب والنباتات الدرنية وبعض البذور الزيتية مثل بذور الكتان linseed. تعطي السيانوجينات عند هدرجتها مركبات سامة مثل سيانيد الهيدروجين hydrogen cyanide ومركبات أخرى مثل carbonyl compounds والتي تؤثر على عملية التنفس وتسبب توقف القلب (Davies, 1991)، علما بأنها تعتبر مواد غير سامة في حد ذاتها.

في دراسة أجريت على أسماك المبروك الشائع *Cyprinus carpio* وجد أن مستويات النمو في هذه الأسماك تتأثر سلبا عند تناولها أعلافا تحتوي على مصادر بها مركبات السيانوجينات مثل بذور الكتان ونبات الكسافا cassava (Hossain and Jauncey, 1989)، إلا أن هذه المركبات لم تحدث ذلك الأثر في أسماك البلطي النيلي *Oreochromis niloticus* عندما تناولت أعلافا تحتوي على مسحوق أوراق الكسافا (٩,٩ جزء في المليون سيانيد) أو مسحوق الكسافا نفسه (٧١,١ جزء في المليون سيانيد) المجففان بأشعة الشمس (Ng and Wee, 1989).

على الرغم من أن السيانوجينات مركبات مقاومة للحرارة، إلا أنه يمكن التخلص منها أو تقليل نسبة تركيزها في المصادر النباتية عن طريق نقع المصدر النباتي في الماء لمدة ٢٤ ساعة (Borlongan, and Coloso 1994).

المركبات الشبه قلوية Alkaloids :

تعتبر المركبات الشبه قلوية مركبات أيضية ثانوية metabolites تتواجد بكثرة في النباتات. و تعتبر البقوليات التي تحتوي على هذه المركبات مكونات مثالية لأعلاف الأسماك وذلك لإحتوائها على نسب عالية من البروتينات القابلة للهضم (٣٠- ٥٠٪) ومنها على سبيل المثال، بذور الترمس *Lupinus albinus*. إن تواجد المركبات الشبه قلوية في المصادر العلفية يخفض من استهلاك الأسماك لهذه المصادر وذلك راجع إلى تأثير هذه المركبات على الأعضاء الحسية organoleptic (de la Higuera *et al.*, 1988). وبناء على ذلك فإن كفاءة الأعلاف تنخفض عندما تتناولها أسماك التراوت القزحية *Oncorhynchus mykiss* نتيجة لاحتواء هذه الأعلاف على مسحوق بذور الترمس Bangoula *et al.* (1993). إلا أنه تبين في دراسة أخرى أن أسماك التراوت القزحية *Oncorhynchus mykiss* وأسماك التربوت *Psetta maxima* قد تتحمل النسب العالية من مسحوق بذور الترمس المضافة إلى الأعلاف، وقد يعود السبب في هذا الاختلاف إلى الطريقة التي تمت بها عملية تصنيع الأعلاف في كلا الدراستين، حيث وجد أن الأعلاف في الدراسة الأخيرة قد تم تصنيعها بطريقة الإنبثاق وأن نسبة المركبات الشبه قلوية فيها كانت < ٢٠ مجم/كجم (Burel *et al.*, 1998;2000a). ويتضح من هذا، أن الطريقة التي تؤثر بها المركبات الشبه قلوية على الأسماك أو معرفة مسار المركبات الأيضية لهذه المركبات داخل جسم الأسماك غير مفهومة بشكل واضح حتى الآن وذلك راجع إلى قلة الدراسات في هذا المجال، إلا أنه يمكن القول بأن الأسماك لديها القدرة على استهلاك مصادر علفية بها مستويات معتدلة من المركبات الشبه قلوية.

تعتبر طريقة الاستخلاص المائي aqueous extraction من الطرق الفعالة في نزع أو تقليل نسبة المركبات الشبه قلوية في المصادر العلفية. كما تتأثر نسبة المركبات

الشبه قلوية في الأعلاف بارتفاع درجة الحرارة المصاحبة لعملية الإنبثاق. حيث لوحظ أن مسحوق بذور الترمس تعطى نتائج أفضل على أسماك التراوت *Oncorhynchus mykiss* عند تصنيعه عند درجة حرارة ١٤٥ درجة مئوية مقارنة بدرجة الحرارة ١٢٠ درجة مئوية (Bangoula *et al.*, 1993). وتعتبر طرق الانتخاب الوراثي أيضا من الوسائل الناجعة لتخفيض نسبة المركبات الشبه قلوية في المصادر العلفية، حيث تم انتخاب أصناف جديدة من بذور الترمس انخفضت فيها نسبة المركبات الشبه قلوية.

المركبات القليلة التسكر والمركبات العديدة التسكر الغير نشوية

Oligosaccharides and Non Starch Polysaccharides :

تعتبر المركبات القليلة التسكر والمركبات العديدة التسكر الغير نشوية مكونات مهمة في كثير من البقوليات والحبوب (Saini, 1989). ويعود تأثير هذه المركبات على الأسماك إلى ربط هذه المركبات لأحماض العصارة الصفراوية أو إعاقة النشاط الإنزيمي في الجهاز الهضمي (Storebakken *et al.*, 1998). حيث لوحظ إنخفاض في الإستفادة من المغذيات نتيجة لإستهلاك كربوهيدرات فول الصويا في أسماك السلمون الأطلسي *Salmo salar* (Arnesen *et al.*, 1989)، إلا أنه لوحظ أيضا عند إستخدام نفس المصدر، تأثيرا خفيفا للمركبات القليلة التسكر على قدرة أسماك التراوت القزحية *Oncorhynchus mykiss* للإستفادة من البروتين. علما بأن المركبات القليلة التسكر الرئيسية في مسحوق فول الصويا الغير منزوع الدهن هي السكروز (٦- ٧٪) و الرافينوز (١- ٢٪) والستاشيوز (٥- ٦٪) وهي تكون بمجموعها ١٢- ١٥٪ من محتوى الكربوهيدرات القابلة للإمتصاص.

إن إضافة المركبات قليلة التسكر المستخلصة من فول الصويا إلى أعلاف تحتوي على مسحوق السمك لم تحدث تغيرا في الشكل الخارجي لخلايا الأمعاء (van der Ingh *et al.*, 1991) أو أي تأثيرات أخرى مرتبطة بالنمو أو بمعامل الهضم

في أسماك السلمون الأطلسي *Salmo salar* (Krogdahl et al., 1995)، يضاف إلى ذلك أن أسماك التراوت *Oncorhynchus mykiss* قد إستفادت بكفاءة من الأعلاف التي احتوت على مسحوق دوار الشمس (Tacon et *al*., 1984; Sanz *et al.*, 1994) ومسحوق فول الصويا (Rumsey *et al.*, 1993; Sanz *et al*., 1994; Kaushik *et al*., 1995). كما أن المصادر العلفية التي تحتوي على مستويات عالية من الكربوهيدرات قد تمت الإستفادة منها من قبل أسماك البلطي *Sarotherodon mossabicus* (Jackson *et al.*, 1982) وأسماك المبروك *Cyprinus carpio* (Ufodike and Matty, 1983).

يعود انخفاض استهلاك الأعلاف في هجين أسماك القاروص المخططة (Gallagher, 1994) وفي أسماك التراوت القزحية *Oncorhynchus mykiss* (de la Higuer *et al*., 1988) وانخفاض معامل الهضم في أسماك التراوت *Oncorhynchus mykiss* (Sanz *et al*., 1994) إلى وجود مستويات عالية من المركبات العديدة التسكر الغير نشوية في أعلاف هذه الأسماك، والتي تتضمن مسحوق فول الصويا ومسحوق الترمس ومسحوق دوار الشمس، على التوالي. وتشمل هذه المركبات البكتينات pectins والجلكتينات galactans والسليلوز cellulose واللجنين lignin. كما أن إرتفاع محتوى الرطوبة في براز أسماك السلمون *Salmo salar* يعود إلى النشاط الإسموزي للمركبات العديدة التسكر الغير نشوية الموجودة في مسحوق فول الصويا (Olli and Krogdahl, 1994; Olli *et al*., 1994b and Refstie *et al*., 1997). كما أن المركبات العديدة التسكر الغير نشوية مثل أرابينن arabinan وأرابينوجلاكتن arabinogalactan والأحماض العديدة التسكر acid polysaccarides والتي تكون ١٤- ١٨٪ من محتوى الكربوهيدرات الكلية في مسحوق فول الصويا الغير منزوع الدهن قد تكون مسؤولة عن ربط المعادن في الأمعاء وإنخفاض معامل هضم الدهون (Storebakken *et al.*, 1998).

يمكن التخلص أو تقليل مستويات المركبات القليلة التسكر أو المركبات العديدة التسكر الغير نشوية عن طريق الطبخ بالبخار، حيث إتضح أن معامل الهضم لكل من الذرة الصفراء والبطاطس في كثير من الأسماك قد تحسن كثيرا. كما أن المعالجة الحرارية لمسحوق الترمس قد حسنت من معامل الهضم للجلاكتينات والمتواجدة بنسبة كبيرة في بذور الترمس (de la Higuera *et al*., 1988). كما تعتبر عملية الإنبثاق extrusion مع الحرارة العالية، أثناء عملية تصنيع الأعلاف، من الطرق التي تعمل على تخفيض مستويات المركبات القليلة التسكر والمركبات العديدة التسكر الغير نشوية، حيث حسنت من هضم الكربوهيدرات في بذور البقوليات (Bangoula *et al*., 1993; Burel *et al*., 2000a)، وذلك بسبب التحطم الشديد لجدران الخلايا والتفكك الجزئي لمركبات ألفا- جالاكتوسيدات -α galactosides.

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Antinutritional Factors in Feed Sources of Plant Origin and Their Effects on Fish: A Review

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Abstract:

The usage of plant sources such as oilseeds, legume seeds, cereals and root tuber meals as fish feed ingredients are limited by the presence of many antinutritional factors. Among the most important of them are protease inhibitors, lectins, tannins, gossypols, phytates, gulcosinolates, saponins, cyanogens, alkaloids and oligosaccharides in addition to non starch polysaccharides. The nature of these factors and their influence on fish are reviewed through maximum and minimum levels of each factor. All studies carried out in this regards were divided into two groups. In the first group the use of pure antinutritional factors which have been extracted from different plants and added to fish feed were considered; whereas the second group deals with plant sources that are known to contain such factors in fish feed. Regardless of the way that these factors have been delt with, different negative consequences such as poor palatability, reduced feed efficiency, low feed digestibility and poor growth have been observed. The effectiveness of treatments to reduce the deleterious effect of these factors in plant sources such as feed processing, heat treatment either wet or dry, aqueous extraction and enzyme treatment have been reviewed for each case.

English Section

القسم العربي

تأثير استئصال المبيض على وزن الجسم ونشاط إنزيم 11β-hydroxysteroid dehydrogenase type I في الكبد وأنسجة الشحم في الجرذان

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الملخص :

إن استخدام استئصال المبيض كنموذج لبحوث ما بعد سن اليأس في العالم واسعة الانتشار، وذلك لتشابه التعقيدات والمشاكل المترتبة على نقص هرمون الاستروجين بسبب أما استئصال المبيض أو بلوغ سن اليأس الطبيعي. تهدف هذه الدراسة إلى التعرف على تأثير استئصال المبيض على نشاط إنزيم 11β-hydroxysteroid dehydrogenase type I ، وبالتالي على وزن الجسم. إثنان وثلاثون من إناث الجرذان عمرها ٦ أشهر استخدمت في هذه التجربة. قسمت الحيوانات إلى مجموعتين، الأولى طبيعية والثانية مستأصلة المبيض مبينة على دراسة قصيرة وأخرى طويلة المدى. تم استئصال المبيضين تحت تأثير المخدر من الناحية السفلى لبطن الحيوان. وتم تسجيل وزن الحيوانات شهريا. في نهاية التجربة تم قتل الحيوانات وأخذ عينات من الكبد وأنسجة الشحم لإخضاعها لفحص النشاط الحيوي، أظهرت النتائج حدوث زيادة حقيقية ($p<0.55$) في وزن الجسم ونشاط إنزيم الكبد. أما في أنسجة الشحم فلم تظهر النتائج فروق حقيقية. تشير هذه النتائج إلى أن استئصال المبايض تساهم في زيادة وزن الجسم ونشاط إنزيم 11β-hydroxysteroid dehydrogenase type I في الكبد مع احتمال ذلك في أنسجة الشحم.

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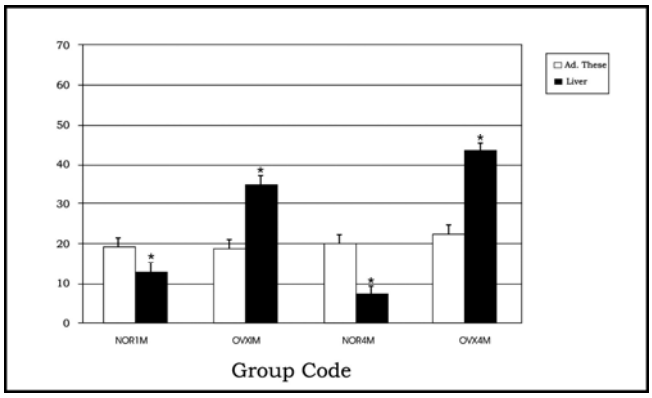

Figure (4) : Comparison between 11β-hydroxysteroid dehydrogenase type 1 (11β-HSDI) activity in the liver and adipose tissue. In the short-term study, (one month after ovariectomy – NOR1M & OVX1M) as well as in the long-term study, (four months after ovariectomy - NOR4M & OVX4M), there was a significant increase in hepatic 11β-HSDI activity. In the adipose tissue, no significant difference detected between the groups. (*, # significant at $P = 0.05$)

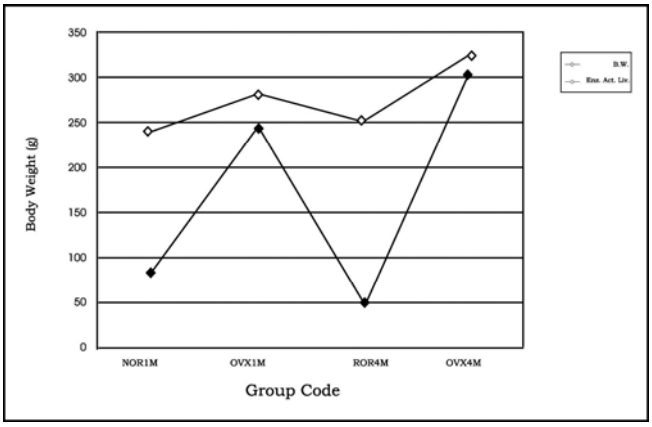

Figure (5) : The relationship between body weight and hepatic 11β-hydroxysteroid dehydrogenase type 1 (11β-HSDl. The graph shows that the significant increase in body weight was associated with a significant increase in 11β-HSDI activity. This was clear in the short-term study (one month after ovariectomy – NOR1M & OVX1M) as well as in the long-term study (four months after ovariectomy - NOR4M & OVX4M). These results suggested the existence of a direct relationship between the two parameters. In the short-term study, as well as in the long-term study,

namely estrogen as it is well established that OVX cause estrogen deficiency, (Eskin *et al*., 2003). Estrogen was reported to be the prime hormone responsible for the postmenopausal changes. It also has been found to influence eating behavior and cause weight gain in animals, (Heymsfield *et al*. 1994). In this study, although food intake was not calculated, the increased food intake was observed in all of the ovariectomized rats.

The effect of ovariectomy on hepatic 11βHSD-l activity in the liver and adipose issue was investigated. It was recorded that hepatic enzyme activity was significantly elevated after ovariectomy. The increase in 11-βHSDl activity in the liver is consistent with the results reported by *Low* and his group (Low *et al.*, 1993) where they reported that gonadectomy resulted in a marked increase in 11β-HSD activity in female liver. Our finding here contradicts the finding by Stewart, and his group (Stewart *et al*., 1999) where they reported hepatic 11β-HSDl is reduced in obesity which was explained to be part of the compensatory change to reduce the local intrahepatic glucocorticoid load.

The elevation in hepatic 11β-HSDl activity following ovariectomy could again be attributed to the metabolic changed cause by ovariectomy-induced estrogen deficiency. In adipose tissues, in the short-term study, OVX was not shown to alter 11β-HSDl activity. The percentages recorded for both groups were almost equal. In the long-term study, OVX was shown to induce a slight increase in 11β-HSDl activity. Although the difference was not found to be statistically significant, it is consistent with Rask *et al.*, (2001), who reported that adipose tissue from obese humans has increased 11β-HSDl activity, which again supports the menopause role in fat re deposition.

Conclusion:

These results suggest that in rats, ovariectomy-induced estrogen deficiency have induced an increase in body weight as a consequence of increasing visceral fat deposition. It also has an effect in modulating 11β-HSDl activity in the liver and in adipose tissue.

18.83 ± 6.41 % for the OVXlM and 18.59 ± 7.09% for the NOR4M compared to 22.39 ± 12.57% for the OVX4M. Generally; hepatic 11β-HSDl activity was lower than the activity in adipose tissue in both the normal groups (NORlM & NOR4M), whereas the opposite is true for the OVX groups (both OVXlM & OVX4M). Figure 4, shows enzyme activity ± standard deviation (SD) for the four groups.

Body weight and 11β-HSD-l Activity: The increase in body weight one month after OVX was associated with a significant increase *(P<0.05)* in enzyme activity when comparing NOR1M and OVX1M on one hand and NOR4M and OVX4M in the other hand. The results suggest direct relationship between body weight and enzyme activity in the liver. Figure 5 gives illustration of the relationship between body weight and enzyme activity in the liver.

Discussion :

This study investigated the effect of ovariectomy (OVX) on body weight and on the activity of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in the liver and adipose tissue. The physical examination of the ovariectomized rats revealed increased abdominal/visceral fat deposition four months after OVX, which was apparent in the OVX4M group. This observation is consistent with the finding reported by Heymsfield *et al*., (1994) and Poehlman *et al*., (1995), which have also, suggest that menopause increases central adiposity. It is also consistent with the view that menopause is associated with changes in fat distribution and that is estrogen deficiency is believed to play a role in postmenopausal women obtaining the gynoid (metabolic syndrome of overweight or obese men) fat deposition, (Reubinoff *et al*. 1995).

Consistent with the physical appearance, ovariectomy was found to induce a significant increase in rats' body weight. The increase in body weight was a clear sign that the rats were entering the menopausal stage as weight gain is one of prime consequences of menopause. The effect of OVX persisted to four months after as the increase in body weight of the OVX-rats were statistically significant when compare to the normal group through out the duration of the study. The increase in body weight following OVX could be explained by decrease in ovarian hormone levels

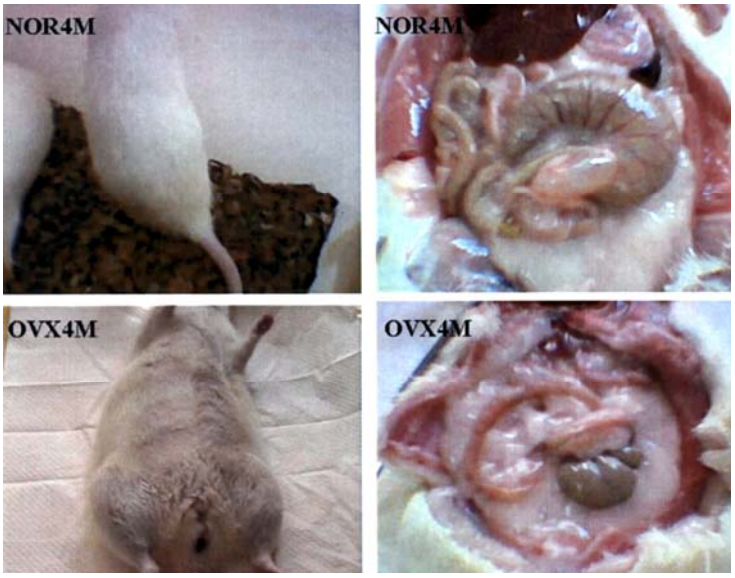

Figure (3) : Illustration of the increased amount of the visceral fat observed in the log-term study in the ovariectomized rats (OVX4M) compared to the normal rats (NOR4M) group. Ovariectomy induced the rate of adiposity that resulted in excessive visceral fat deposition and in turn body weight. The main body weight (±SD) for the NOR4M group was 255.73 ± 35.82 compared to 324.91 ± 47.37 for the OVX4M group.

The Effect of Ovariectomy on 11β-HSD1 Activity: In the both the short and long-term studies; OVX induced a significant increase *(P<0.05)* in the rate of conversion of corticosterone (B) to ll-dehydrocorticosterone (A) in the liver. The mean enzyme activity recorded for OVX1M (34.95 ± 21.67%) is significantly higher than the activity recorded for the NORlM (12.8 ± 8.04%) group. The mean enzyme activity recorded for OVX4M (43.20 ± 21.62%) is significantly higher than the activity recorded for the NOR4M (7.97 ± 4.40%) group.

In the adipose tissue the test showed no significant differences in both the short and long-term studies although the OVX4M showed a slight increase of 3.8% when compared to the NOR4M group. The following values were recorded for each group: 19.36 ± 3.73% for the NOR1M compared to

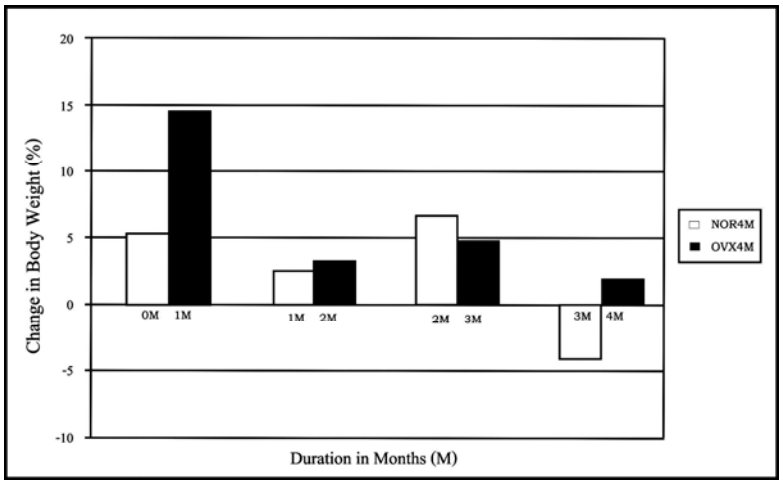

Figure (2) : Percentage changes in body weight recorded in the long-term study, (four months after ovariectomy) for the normal (NOR4M) and ovariectomized (OVX4M) groups throughout the duration of the study.

In the first month following OVX, there was an increase of 14.6% in the OVX4M group compared to 5.2% increase in the NOR4M group. In the second month, there was an increase of 2.9% in the OVX4M group compared to 2.3% in the NOR4M group. In the third month, the following changes in mean body weight were recorded: 4.6% in the OVX4M group and 6.5% in the NOR4M group. Thus, results at this stage showed less weight gain by the OVX4M group compared to the NOR4M group. In the fourth month, there was a decrease of 4.2% in the NOR4M group, compared to 1.8% increase in the OVX4M group. Percentage changes in body weight recorded for both groups are presented in Figure 2.

The physical examination of the rats: Matching the process of ovariectomyinduced weight gain, all the ovariectomized rats showed large amount of visceral/abdominal fat deposition. Figure 3 illustrates the increase in the amount of the visceral fat deposition in the OVX4M group compared to the NOR4M group as revealed by the physical examination and dissection of these rats.

the other hand, the OVX1M; ovariectomy induced a significant increase *(P<0.05)* in mean body weigh one month after ovariectomy. The mean body weight of those rats increased from 253.8 ± 25.6g to 282.3 ± 25.3g. Figure 1 illustrates the mean value of body weight at base line and one month after ± standard deviations (SD) for both groups.

In the long term study, (four months after ovariectomy), the statistical ANOVA of these groups showed significant increase *(P<0.05)* in MBW of the OVX4M between base line (259.32 ± 27.30g) and four months after OVX (324.91 ± 47.37g), whereas no significant difference recorded for the NOR4M. MBW of this group increased from 226.90 ± 30.77g to 255.73 ± 35.82g.

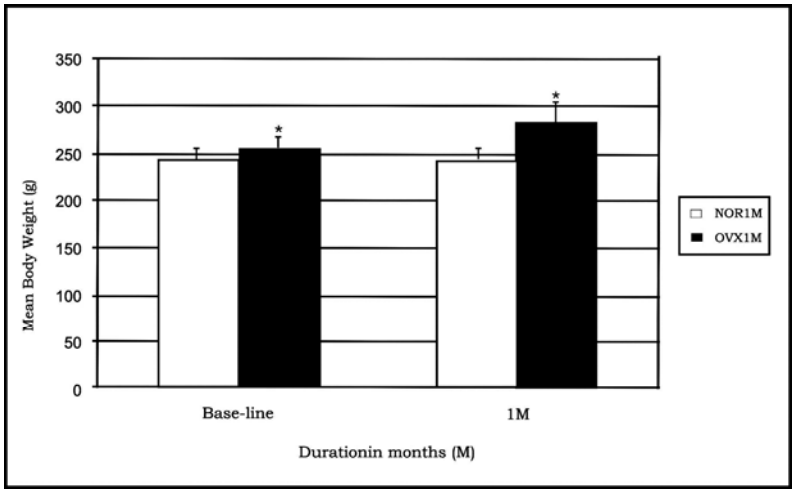

Figure (1) : Comparison of rats body weights recorded in the short-term study, (one month after ovariectomy) normal (NOR 1 M) and ovariectomized (OVX1M) rats. Ovariectomy resulted in significant increase (*, P<O.05) in mean body weight of the OVX1M group one month after compared to the mean body weight at base-line. On the other hand, results showed no significant difference in mean body weigh of the NOR1M group at baseline and one month after. Values are mean ± SD.

divided into OVX1M and OVX4M. To avoid the risk of infection post surgery, rats were housed in soft tissue bedded cages and the wounds were sprayed daily with a surgical disinfectant for a period of one week.

Tissue harvesting and preparation: Rats were sacrificed by cervical dislocation either one month or four months after OVX as allocated. Immediately upon sacrificing, tissue samples from liver and adipose tissue were collected and dissected on ice to minimize disturbance to the enzyme's activity within the tissues. Tissues were then stored at into -70°C environment for bioactivity assay.

11β-HSDl Activity in the Liver and Adipose Tissue: Tissues samples were thawed and homogenized in 2 ml of Krebs-Ringer buffer + 0.2% glucose at pH 7.4 in the same day of the assay. Homogenates were assayed for protein content calorimetrically (Bio-Rad, Hercules, DA, USA). Protein homogenate 80µg/ml protein for liver sample, (Moisan *et al*., 1990) and 750µg/ml protein for adipose tissue sample, (Lindsay *et al.*, 2003) was added to 2.5µl [^{3}H] corticosterone with 20 mM NADP. The total assay volume was made up to a total volume of 250 µl by adding Krebs-Ringer buffer. The final volume was than incubated at 37°C either 30 minutes (liver) or overnight for adipose tissue. Assays were in the dehydrogenase direction (corticosterone (B) to 11-dehydrocorticosterone (A), which is more stable in tissue homogenates. The reaction was terminated by addition of ethyl acetate. Steroids was separated by TLC using chloroform:ethanol (95:5, vol:vol), visualized under UV light and quantification by scintillation counting using β counter.

Statistical Analysis: Data were analyzed by T-test and one-way ANOV A. When the main effect was significant, a post-hoc test (Tukey) was applied to determine individual differences between means. A value of *(P<0.05)* was considered significant.

Results:

The Effect of Ovariectomy on Body Weight: In the short-term study, (one month after ovariectomy), the statistical T-test of these two groups showed no significant difference *(P=0.05)* in mean body weight (MBW) of the NOR1M one month after (from 244.7 ± 24g to 243.1 ± *20Ag).* On

Glucocorticoids are a group of corticosteroids that affect carbohydrate metabolism by inducing liver gluconeogenesis, glycogenolysis and thus elevation of blood sugar (Allary & Annane, 2005). They also play a role in fat and protein metabolism, maintenance of arterial blood pressure and alteration of the connective tissue response to injury. Glucocorticoids have been shown to potentiate the adipogenic process and anabolic lipid metabolism in adipocytes (Berger *et al.*, 2001).

This study aimed to investigate the effect of OVX on body weight and on the activity of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in the liver and in adipose tissue.

Materials and Methods:

In this set of experiments two main things were investigated; first, the effect of ovariectomy (OVX) on body weight and second, the effect of OVX on the activity of the enzyme l1β-hydroxysteroid dehydrogenase type 1 (11β-HSDl) in the liver and adipose tissue.

Animals: Thirty Adult female (Sprague Dawley) rats, 6 months old were used in the experiment. The rats were housed at normal room temperature with adequate ventilation and normal 12-h light- dark cycle with free access to food (commercial laboratory rat's food) and water. Based on the allocated timing for sacrificing, the study was divided into short-term and long-term. In the short-term study two groups were included, the normal NOR1M (n = 8) and the ovariectomized OVX1M (n = 8) which were sacrificed one month (1 M) after the beginning of the study. In the long-term study, the normal NOR4M (n = 8) and OVX4M (n = 8) which were included were sacrificed four months (4M) after the beginning of the study. The rat's body weight was monitored and recorded monthly. This work has the approval of the Animal Ethics Committee at the Institute for Medical Research - Malaysia.

Ovariectomy: For ovariectomy (OVX), sixteen rats were anesthetized with intramuscular injection (IM) of Zoletil 50 O.lml (Virbac Laboratories, France), Ketamav O.lml (MA VLAB, Australia) and Xylazil 0.03 ml (Troy Laboratories, Australia). OVX was performed using the ventral approach. Upon recovery from anesthesia, animals were randomly

healing (Qiao *et al*., 2005), osteoarthritis and cartilage abnormalities (Cake *et aI*., 2005) fat and lipid metabolism (Kamei *et al*., 2005), 11β-hydroxysteroid dehydrogenase (11β-HSD) activity in adipose tissue, liver, kidney and brain (Low *et al*., 1993) and the relationship between the oral discomfort (Seko *et al*., 2005).

Postmenopausal women are at high risk of weight gain, especially those already characterized by increased weight and fat mass (Dubnov *et al*., 2003), coronary artery diseases and stroke, as well as of osteoporosis and fractures (Sowers & La Pietra, 1995). Weight gain at menopause increases the risk of high blood pressure, high blood lipid levels, and insulin resistance. Alterations of lipid and carbohydrate metabolism are deeply related to increased risk of cardiovascular mortality and morbidity (Gaspard *et al*., 1995).

The link between obesity and the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSDI) has been investigated by different studies. *in vivo* studies have shown that adipose regeneration of cortisol is enhanced in human obesity (Sandeep *et al*., 2003). Rask *et al*., (2001) reported that adipose tissue from obese humans has increased 11βHSD-I activity. In another study it was also shown that omental adipose tissue contains significantly more 11βHSD-I activity than subcutaneous adipose tissue into adipocytes by cortisone compared to those from the subcutaneous adipose tissue (Berger *et al*., 13).

11β-HSD1 plays an important role in determining intracellular glucocorticoids concentration by the interconversion of cortisol (F) and cortisone (E) in man and corticosterone (B) and 11-dehydrocorticosterone (A) in rodents. It is one of two identified isozymes of the enzyme 11β-HSD that interconvert hormonally active cortisol and inactive cortisone (Bujalska *et al*. 2002). 11β-HSD1 was first isolated from the liver but it has a widespread central nervous system and peripheral tissue distribution and functions as NADP dependent. Type 2 (11β-HSD2) functions as NAD+ dependent dehydrogenase of adrenal glucocorticoids (Hult *et al*., 1998). Glucocorticoids are 21-carbon steroid molecules with a variety of physiologic and metabolic effects (Schleimer *et al*. 2003) and cortisol (hydrocortisone) is the principal circulating glucocorticoid in humans.

Effects of Ovariectomy on Body Weight and Activity of 11-Beta Hydroxysteroid Dehydrogenase Type I In the Liver and Adipose Tissue of Rats

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Abstract:

The use of ovariectomized rat model in postmenopausal researches is widely used due to the similarities between the complications caused by estrogen deficiency either in ovariectomy or in natural menopause. The aim of the study was to investigate the effect of ovariectomy on the activity of the enzyme 11β-hydroxysteroid dehydrogenase type I (11β-HSDI) and consequently on body weigh. Thirty two Adult female (*Sprague Dawley*) rats, 6 months old were used in the experiment. Rats were divided into normal and ovariectomized groups, on long and short-term studies. Bilateral ovariectomy was performed under anesthesia using the ventral approach and rat's body weight was checked monthly. At the end of the study period, rats were sacrificed and tissue samples from liver and adipose tissue were collected for bioactivity assay. Ovariectomy induced significant increase *(P<O.05)* in body weight as well as in hepatic enzyme activity. In adipose tissue there was no significant difference recorded. These results suggest that ovariectomy has an effect on body weight and in modulating 11β-HSDI activity in the liver and probably in adipose tissue.

Introduction :

An animal model to study the postmenopausal symptoms and complications has long been defined in ovariectomized (OVX) rats. OVX-rats are deficient from ovarian hormones and share similar characteristics with naturally occurring menopause. Thus, the OVX animal model was used to study a list of menopausal complications such as obesity (Saruhan & Ozdemir, 2005), postmenopausal bone loss (Kalu, 1991), fracture

مقارنة فاعلية الدامنيزين، السورامين، القوابايرامين و بروميد الهوميدوم في معالجة فئران مصابة بسلالة *Trypanosoma evansi* المسبب لمرض الهيام (النوم)

حمدان بن إبراهيم المحمد

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المملكة العربية السعودية

المخلص :

يعتبر استخدام أدوية مضادات الطفيليات للوقاية والعلاج أحد الطرق الرئيسية لمكافحة طفيل *Trypanosoma evansi* المسبب لمرض الهيام (النوم). أجريت هذه الدراسة لاختبار تأثير أربعة من الأدوية الشائعة في علاج هذا المرض بين الحيوانات حيث تم استخدام عدد ٢٥ من الفئران السويسرية (مقسمة إلى خمس مجموعات) والتي أمرضت سلفا بحقنها جميعا بجرعات متساوية من سلالة معزولة من طفيل *Trypanosoma evansi*.

حقنت ٤ مجموعات في التجويف البروتوني بالأدوية وهي كالتالي: المجموعة الأولى استورات الدامنزين (بيرنيل) بجرعة مقدارها ٣,٥ مليجرام للكيلوجرام، المجموعة الثانية سورامين (نجانول) بجرعة مقدارها ١٠ مليجرام للكيلوجرام الواحد، المجموعة الثالثة القوابايرامين (انتراسيد) بجرعة مقدارها ٥ مليجرام للكيلوجرام, أما المجموعة الرابعة فحقنت بمركب بروميد الهوميدوم (بروميد الايثديم) بجرعة مقدارها ١ مليجرام للكيلو جرام, وتركت المجموعة الخامسة بدون علاج كضابط للاختبار.

هذا وقد تم شفاء الفئران من الإصابة بواسطة مركب النجانول بعد يومين من بدء العلاج أما البيرنيل فتم الشفاء بعد ثلاثة أيام إلا أن البيرنيل تسبب في موت ٢ من ٥ من الفئران المعالجة في اليوم التالي لبدء العلاج بينما لم يتسبب النجانول في موت أي من الفئران. ولقد فشلت بقية الأدوية في شفاء المرض وربما تسببت في آثار سمية في الفئران.

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Antrycide, on the other hand, showed much toxicity to mice after I.P. administration and cause death of all five animals. Swiss Webstar mice did not tolerate a single-dose regimen of 0.5 mg/kg antrycide which appeared to be in the borderline for acute toxicity. Singh *et al*., (1993) used antrycide in treating *T. evansi* in infected dogs with complete recovery of two dogs, while another dog died on the day therapy was initiated. These results agreed with Uilenberg (1998) who described toxicity problem of antrycide in cattle and horses. It is apparent that the toxicity of drugs differs in different species of animals. Much controversy results could be noticed about the efficiacy of the drug in both *in vivo* and *in vitro* studies. Kaminsky and Zweygarth (1989) reported that care must be taken when evaluating anti-trypanosomal drugs for in vitro potency because drugs might be inactive in the *in vitro* system but still be efficacious *in vivo*.

Because of its low toxicity margin and the inability to use it at higher dosage rates, antrycide has lost its popularity for use against *T. evansi.*

Collectively, the data presented indicate that suramine followed by berenil are the best trypanocidal drugs for *T. evansi*. Antrycide should be avoided due to its major toxic side effects.

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Author thanks Dr. Faisal M. Abu-Tarboush (King Saud University, Riyadh, SA) for the gift of mice.

Discussion:

Drug control of animal trypanosomiasis relies essentially on three drugs, namely: Ethidium bromide, Berenil ac eturate and Naganol. More recently Antrycide has been reintroduced because of the need to especially combat camel trypanosomiasis. The results obtained from this study clearly demonstrated that Naganol and Berenil were powerful antitrypanosomal compounds with a specific activity *in vivo* comparable to Ethedium bromide. Both drugs are curative 100% in model trypanosome infections and were effective in animals with heavy parasite burden (range of parasitaemia was 60-350 and 200-300 parasites, respectively). Importantly, the toxicity of Naganol was very low if compared with Berenil which caused death in 2/5 of experimental animals.

Berenil was reported by Tuntasuvan *et al*., (2003) to cause mild to severe toxicity in horses and mules after injection, with minimal protective effect of the drug. Berenil was proved to be effective for the treatment of surra in cattle, buffalo, sheep, pigs and camels (Peregrine and Mamman 1993), but was reported to cause fatal reactions in camels, horses and dogs at doses which are considered to be normal and harmless in cattle (Sirivan *et al.* 1994).

Although showed toxicity and death of 3/5 of animals, Ethidium bromide had no therapeutic action on the remaining two animals of this study indicating that the strains of *T. evansi* isolated in Al-Ahsa might be resistant to that drug; a serious problem in the chemotherapy of Surra. Although the exact mechanism of drug resistance is insufficiently known, two mechanisms have been proposed by Uilenberg (1998); adaptation and selection theories. Impairment of the host immune system may lead to the rapid development of drug resistance by *T. evansi* under experimental conditions in mice (Osman *et al.* 1992). Reports of drug resistant *T. evansi* (or even multiresistant strains) are emerging from allover the world (Brun and Lun 1994, WHO 1998). Care must be taken in reporting drug resistance since the inaccessibility of the drug to tissue stages of trypanosomes or the insensitivity of some stages in the life-cycle of the trypanosome to the drug could be the reason (Jennings *et al*. 1977).

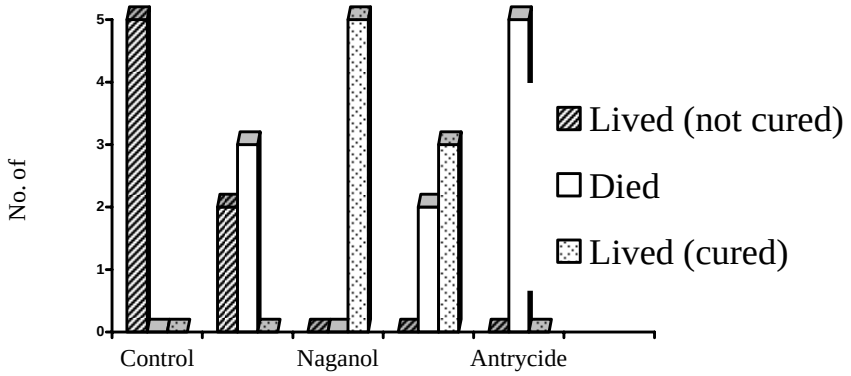

Figure (1) : Effect of four Trypanocides on *T. evansi* infected mice.

Results:

The effect of antitrypanosomal chemotherapy is shown in table (1) and figure (1). In the control group, parasitaemia showed the usual undulating course. In Ethidium bromide treated mice, three mice died next day of treatment (day 16). The other two mice showed parasetaemia not statistically different from control group with no effect of the drug on the parasites. In Naganol treated mice, the five animals showed dramatic response to the drug and clearance of parasetaemia occurred on the second day of chemotherapy. The mean number of parasites in blood smears on the day of chemotherapy was 242 that dropped on the next day to a mean of 42 followed by complete clearance of parasetaemia on the second day (day 17). In Berenil treated mice, two animals died next day of chemotherapy and the other three mice responded well to treatment. The mean number of parasetaemia on the day of therapy was 270 that dropped to a mean of 113.3 on the first day, 55.6 on second day and complete cure on third day of therapy (day 18). In Antrycide treated mice, all five mice died next day of I.P. chemotherapy.

Drugs: Drugs were dissolved and prepared as aliquots (according to manufacturer's instructions), to be injected intraperitoneally (I.P.) in the following concentrations: Diminazene aceturate (Berenil, Hoechst, Germany): 3.5 mg/kg, Suramine (Naganol, I.C.I., UK): 10.0 mg/kg, Quinapyramine (Antrycide, Bayer, Germany): 5.0 mg/kg and Homidium bromide (Ethidium bromide, May and Baker, UK): 1.0 mg/kg.

In vivo drug activity- each mouse was inoculated I.P. with 10^4 parasites and infection was allowed to develop for 14 days when treatment was initiated on day 15. Mice were checked daily (one day before therapy and thereafter for 17 days) for parasitaemia in blood collected from tail vein, (study period = 31 days). Animals were considered cured when no trypanosomes were detected during the 17 days of observation period (Bacchi *et al*. 1998).

Table (1)

State of parasitaemia in mice infected with *T. evansi* after treatment with four trypanocides.

days	Mean No of parasites in blood smear																	
Control	14	15	16	17	18	19	20	21	21	23	24	25	26	27	28	29	30	31
	183	147	79	33	0	12	52	93	137	214	0	0	25	59	214	157	104	98
	0	8	43	83	117	135	168	205	222	269	341	417	399	325	298	189	150	131
	121	200	254	290	305	317	261	214	138	94	55	34	20	0	0	13	75	115
	253	194	103	88	37	15	0	0	0	1	7	28	50	113	147	203	225	270
	55	76	100	106	127	201	232	163	105	97	38	4	0	2	36	85	133	190
Ethidium bromide	172	180	173	190	200	0	0	2	26	163	178	231	157	192	214	250	194	167
	9	12	45	230	300	325	398	413	215	176	50	0	0	150	97	45	3	0
	230	400	x															
	35	130	x															
	320	400	x															
Naganol	40	60	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50	300	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	200	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	158	300	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	311	350	95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Berenil	150	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	300	210	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	130	200	x															
	200	300	x															
	400	350	130	70	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Antrycide	170	320	x															
	15	75	x															
	87	200	x															
	40	110	x															
	100	230	x															

X = animals died

active drugs are available, animals have to rely on their own immune defenses alone to combat the disease (Uilenberg 1998).

Chemotherapeutic drugs disrupt or block one or more of the vital processes which are essential to the parasite. Some compounds have specific effects on some enzyme system or block essential metabolic pathways. The exact way in which they work is often not known or only incompletely understood (Zhang *et al*. 1991). Chemotherapy, by stopping the multiplication of the trypanosomes, helps the immune system to overcome the infection (Osman *et al.* 1992). Chemotherapeutic drugs are toxic to the trypanosomes and often have a similar disruptive effect on the cells of the host (Jennings *et al.* 1977), and so should always be used with care and at the recommended dose level only (Homeida *et al*. 1981). It is estimated that in Africa 25-30 million doses of trypanocidal drugs are used annually in the treatment of animal trypanosomiasis, but the population of animals at risk indicated that ten times this figure were necessary (Ilemobade and Buys 1970).

Many investigators have reported therapeutic trials of *Trypanosoma evansi* with the use of different chemotherapeutic drugs (Homeida *et al*. 1980, Bacchi *et al.* 1998, Tuntasuvan *et al.* 2003). This study was done to compare *in vivo* action of the four commonly used chemotherapeutic drugs (Diminazene, Suramine, Quinapyramine and Homidium Bromide) on mice infected by locally isolated *T. evansi*.

Materials and Methods:

Trypansome: a cryopreserved strains of *T. evansi* originally isolated from naturally infected camels in Al-Ahsa Area, Saudi Arabia, that were propagated in laboratory bred rats, were extracted, purified and adjusted to yield 10^4 parasites (Al-Mohammed 2006) were used for infection of experimental animals.

Animals: Twenty-five female Swiss Webstar mice weighting 20-25gm of each were used for *in vivo* drug tests. Mice (groups of fives) were treated with four antitrypanosoma drugs at the appropriate concentrations and one group of animals was used as control.

Comparative *In-Vivo* Activities of Diminazene, Suramine, Quinapyramine and Homidium Bromide on *Trypanosoma evansi* infection in Mice

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Abstract:

Chemotherapy and chemoprophylaxis are the main methods of trypanosomal control. This study was done to compare the *in vivo* efficacy of four commonly used antitrypanosomal drugs. Twenty five Swiss Webstar mice (groups of fives) infected with locally isolated *Trypanosoma evansi* strains were used. Four groups were intraperitoneally injected by therapeutic doses of Diminazene aceturate (Berenil): 3.5 mg/kg, Suramine (Naganol): 10.0 mg/kg, Quinapyramine (Antrycide): 5.0 mg/kg and Homidium Bromide (Ethidium Bromide): 1.0 mg/kg. The fifth group of mice was used as a non-treatment control. Animals with heavy parasitic burden were cured by both Naganol and Berenil after 2 and 3 days of therapy, respectively. Unfortunately, Berenil caused death in 2/5 of experimental animals next day of therapy while Naganol showed no detectable toxic effects. Other drugs either failed to cure the infection or produced toxic effects in animals. In conclusion, Naganol is recommended for treatment of *Trypanosoma evansi* infection of mice.

Introduction:

Trypanosomiasis has continued to disrupt human life, animal husbandry and wild life in most parts of the world (Kuzoe 1993). The field control of animal trypanosomiasis has, over the years, relies on two broad strategies: using chemotherapeutic agents on infected animals and vector control. In general, however, the chemotherapeutic approach is used much more widely than vector control because it is easier to kill the trypanosomes than the flies (WHO 1998). Current methods of treatment of trypanosomes are still unsatisfactory because the number of available drugs is limited and the treatment is usually associated with severe side effects (Kaminsky and Brun 1998). The emergence of drug resistant trypanosomes implies failure of treatment or prevention, and if no other

إمكانية منع الإسهال في الفئران بعد حقن المستخلص المائي لأغاريض نخيل التمر

عبدالله بن يوسف الطاهر

قسم وظائف الأعضاء والكيمياء الحيوية والأقربازين
كلية الطب البيطري والثروة الحيوانية - جامعة الملك فيصل
الأحساء – المملكة العربية السعودية

الملخص :

تعتبر شجرة نخيل التمر من أقدم الأشجار زراعة في العالم. هناك عرف شائع بين الناس والعطارين ان المستخلص المائي لأغاريض النخيل له فوائد في علاج المغص والإسهال. ولتوضيح صحة هذه الادعاءات العلاجية عملت دراسة لبيان تأثير ماء اللقاح على الجرذان المعالجة بزيت الخروع حيث يمتلك القدرة على العمل كمسهل ويزيد من الحركة الدودية و كمية السوائل في الأمعاء وهذا مثبت علميا. وبعد إجراء التجارب تبين من نتائج الدراسة أن ماء اللقاح له نتائج ايجابية في التقليل من حدة الإسهال والحركة الدودية للأمعاء. حيث قلل ماء اللقاح إحصائيا وبشكل معنوي من حدة الإسهال والحركة الدودية للأمعاء وهذا يؤيد رأي العطارين ويسنده لكن لم يكن له تأثير علي زيادة كمية السوائل في الأمعاء. تعتبر هذه الدراسة مبدئية وتحتاج إلي دراسات أخرى للتعرف على المركبات الكيميائية التي يحتوي عليها ماء اللقاح وتأثيرها الدوائي.

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atropine, It was noted that the extract did not affect castor oil induced intestinal fluid accumulation and the volume of intestinal content.

In this study, atropine and the extract produced a significant reduction in the mean number of defecation and increased intestinal transit time possibly due to its anti-cholinergic effect (Brown and Taylor, 2000). However, they did not inhibit castor oil induced enteropooling. This may suggested that mediators other than acetylcholine are involved in castor oil induced enteropooling. It was suggested that Castor oil and its active metabolite ricinoleic acid produce diarrhoea by diminishing intestinal sodium and cholride absorption (Gaginella and Phillips, 1975; Luderer *et al.*, 1980). An increase in intestinal transit time with atropine could also result from reduction in gastric emptying (Izzo *et al.*, 1999a. 1999b).

The results of this study reveal that the aqueous sapthe extract of *Phoenix Dactylifera L* contains pharmacologically active substance(s) with antidiarrhoeal properties. These properties may explain the rational for the effective use of the plant as an antidiarrhoeal agent in traditional medicine. Further study, however, is necessary to isolate and identify the active ingredients of spathe and their precise mechanism of action.

Acknowledgment:
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3.3. Gastrointestinal transit effect

Phoenix Dactylifera L spathe extract significantly (P<0.01) decreased the distance traveled by marker and consequently the percentage of intestinal transit in a dose dependent manner. The three doses of the extract (3, 6, 12 mg/kg) produced 75.32±2.11, 68.57±3.94 and 54.27±3.54% intestinal transit induced by castor oil, respectively. However, atropine (3 mg/kg, i.p.) exhibited much more marked reduction (28.64±4.84%).

Table (3)

Effect of *Phoenix Dactylifera L* extract on castor oil induced small intestine transit in rats

treatment	Dose (mg/kg p.o.)	Total length of intestine (cm)	Distance traveled by marker (cm)	%intestinal transit
Saline	2 ml	119+0.89	109.66±3.23	92.16±2.61
Atropine sulfate (3 mg/kg i.p.)	----	122.2+5.85	35.6±7.03***	28.64±4.84***
Spathe extract	12	126.14+1.7	66.86±3.54***	54.27±3.54***
Spathe extract	6	119.16+0.83	81.66±4.59***	68.57±3.94***
Spathe extract	3	119.5+0.96	90.0±2.58*	75.32±2.11**

Effect of date palm spathe extract on castor oil-induced small intestine transit in rats. Extract was administered 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiments. *$P<0.01$, **$P<0.001$, ***$P<0.0001$ when compared with castor oil and saline treated group.

4- Discussion

The aim of the present study was to assess the effect of an aqueous extract of *Phoenix Dactylifera L* spathe against diarrhoea using experimental diarrhoea models in rats.

The present results show that *Phoenix Dactylifera L* spathe aqueous extract produced a statistically significant reduction in both Co induced intestinal transit and frequency of diarrhoea effects in rat. Consistent with

dose-dependent (19-42%) inhibition of the severity of diarrhoea induced by castor oil was observed (Table 1).

Table (1)

Effect of date palm spathe extract on castor oil induced diarrhoea in rats

Treatment	Dose (mg/kg p.o.)	Mean of defecation	%inhibition of defection
Saline	2 ml	12.333±0.71	------
Atropine sulfate (3 mg/kg i.p.)	----	4.8333±0.31***	61
Spathe extract	12	7.1666±0.4***	42
Spathe extract	6	8.3333±0.42***	32
Spathe extract	3	10±1.0	19

Effect of date palm spathe extract on castor oil-induced diarrhoea in rats. Extract was administered 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiment. **P<0.01, ***P<0.0001* when compared with castor oil and saline treated group.

3.2. Castor oil-induced enteropooling

Dactylifera L spathe extract did not show any significant effect on the intestinal fluid accumulation induced by castor oil when compared with the saline control group. Atropine sulfate, the reference drug, gave a better activity, with 26% of suppressive but non significant effect (Table 2).

Table (2)

Effect of *Phoenix Dactylifera L* spathe extract on castor oil induced enteropooling in rats

Treatment	Dose (mg/kg p.o.)	Mean Wt. intestinal content	%inhibition Wt. intestinal content
Saline	2 ml	2.65±0.3	-------
Atropine sulfate (3 mg/kg i.p.)	----	1.96±0.15	26
Spathe extract	12	2.15±0.12	19
Spathe extract	6	2.45±0.62	7
Spathe extract	3	2.33±0.2	12.2

Effect of date palm spathe extract on castor oil-induced enteropooling in rats. Extract was administered orally 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiments.

for 4 h. The mean±SE of the stool for 4 h of treated groups was compared with that of control group (saline group).

2.4.2. Castor oil-induced enteropooling

Intra-luminal fluid accumulation was determined by the method of Robert et al., (1976). Five groups of 6 animals each fasted overnight were used. Group 1 served as control and was given saline (2 ml/kg i.p.) followed by Co (1ml p.o.) 1h later. The second group was given atropine sulphate (3 mg/kg i.p.) followed by Co. The last three treated groups were given spathe extract (12, 6 and 3 mg/kg p.o.) 1h before Co. After 2 h the rats were sacrificed. The two ends of intestine were tied with thread. The intestine was removed and weighed. The intestinal content was removed by milking. The intestine was reweighed and the difference between full and empty intestine was calculated

2.4.3. Gastrointestinal transit test

Overnight fasted rats (18 h) were divided into five groups (6 rats each). Spathe extracts (12, 6 and 3 mg/kg p.o.), saline (2 ml/kg i.p.), and atropine sulfate (3 mg/kg i.p.) were given 1h before Co. One ml of marker (10% charcoal suspension in 5% gum acacia, Arabic gum) was given orally 1h after Co. The animals were killed by cervical translocation after 1h of marker administration. The distance traveled by charcoal meal from pylorus to caecum was measured. The result was expressed as a percentage of distance traveled by charcoal meal/total distance from pylorus to caecum (Mascolo et al., 1994).

2.5. Data analysis

Results are expressed as mean ±S.E.M and presence of significant differences among means of the groups was determined using one way ANOVA with a Tukey-Kramer post-test for significance. Values were considered significant when $P<0.05$.

3- Results:

3.1. Castor oil-induced diarrhoea

Dactylifera L spathe extract significantly ($P<0.01$) inhibited the mean number of defecation when compared to saline group, and produced a

abdominal gases and pain especially after heavy meal and claimed to have anti-spasmodic activity. The objective of this study was to investigate the possible enteropooling, antidiarrhoeal and intestinal transit time effects of the date-palm spathe extract in rats.

2- **Materials and methods:**

2-1 Plant materials

2-1.1 Preparation of the extract

During the pollination season, the hard envelopes, spathes left behind were directly purchased from local farm. The spathes were cleaned, air dried, cut into small pieces and pulverized into powder with grinder. About 100 g of the powder was packed into thimble and extracted with water in soxhlet apparatus for 3 h. the water extract yield was 10% w/w which was used for pharmacological screening.

2.2. Animals

Male albino rats weighing between 200 and 250 g, obtained from King Saud University, Saudi Arabia, were used. They were housed in wired cages in the animal house at 24±2 °C and relative humidity 44–56%, light and dark cycles of 12 and 12 h, respectively, for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and water was allowed *ad libitum*. Spathe aqueous extract in an amount of 3 mg, 6 mg and 12 mg were used.

2.3. Drugs

Castor oil, atropine sulphate, charcoal, *Acacia nilotica* (Arabic gum); were obtained from Sigma, USA.

2.4. Pharmacological studies

2.4.1. Castor oil-induced diarrhoea

Five groups of animals (6 rats each) were housed in separate cages having paper placed below for collection of faecal matters. Diarrhoea was induced by oral administration of castor oil (Co; 1 ml/rat, p.o.) (Awouters *et al.*, 1978). Group 1 served as control and received saline (2 ml/kg i.p.). The second group received atropine sulphate (3 mg/kg i.p.) as standard. Groups 3–5 were given the test extract (12, 6 and 3 mg/kg p.o.) 1 hr before Co. The number of both dry and wet droppings was counted every hour

Possible anti-diarrhoeal effect of the date palm (*Phoenix Dactylifera L)* spathe aqueous extract in rats

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Abstract:

The date palm (*Phoenix Dactylifera L.*) is known to be one of the oldest cultivated tree in the world. Date palm spathe aqueous extract has claimed to have anti-spasmodic activity. Castor oil-induced diarrhoea, enteropooling and gastrointestinal transit test were elucidated in rats. The results show that aqueous extract significantly reduced both castor-oil induced intestinal transit and frequency of diarrhoea effects. However, the extract did not affect castor oil induced intestinal fluid accumulation. Further studies are needed in regard to isolation of the effective components of these extracts and clarification of its pharmacological mechanisms in the future.

1- Introduction:

The date palm (*Phoenix Dactylifera L.*) is known to be one of the oldest cultivated trees in the world. Its fruit has long been used as a staple food for the native population. The non fruit material is also of great value, as for example, the date palm leaf and the leaflets are utilized for fences, mattings, baskets or hard fans. The fibrous tissue surrounding the base of the date palm leave is a good material for making ropes and fiber for packing (Dowson, 1982). The hard covering (spathe) of the male and female inflorescences is traditionally used by the people of Al-Ahssa, Saudi Arabia as flavoring material for cold and hot drinks because of its characteristic and desirable fragrance. During the pollination season, the hard envelope left behind is cut into small pieces and soaked in drinking water to improve the flavor of water for immediate consumption during summer season. The farmers sometimes boil the wood cuttings in the farm yard for distillation purposes. The liquid distillate obtained is locally known as Maa Al-liqah or Maa Al-Tiltal, water of the spathe. Small private sectors have been started to commercialize the water of the spathe. It is also believed that the distillate has certain medical uses as it relieves

تأثير حقن سموم الاندوتكسن في ضرع الجمال المرضعة

عبدالله محمد الدغيم

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جامعة الملك فيصل، الأحساء، المملكة العربية السعودية

الملخص :

لقد تسبب حقن ١٠ ميكروجرام من سموم الاندوتكسين لبكتيريا الاشريكا القولونية في ضرع الجمال المرضعه في زيادة خلايا النتروفيل في الدم وزيادة الخلايا المتعددة الانوية وكاشف التهاب الضرع وانزيم الاستايل جلكوز اميتريز واللاكتوفرين في الحليب . لقد اقترح بأنه ربما يكون حقن اللاكنوفرين ذا قيمة في علاج التهاب الضرع .

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bacteria, yeasts and moulds. The highly cationic bactericidal domain of lactoferrin located at the N-terminus of the protein increases bacterial cell membrane permeability and causes the release of lipoplysaccharides from Gram-negative bacteria (Ellison *et al*., 1988; Bellamy *et al*., 1992; Yamauchi *et al*., 1993). Besides bacteriostatic and bactericidal activities, lactoferrin may also exert antimicrobial activity through regulation of systemic immune responses by activated neutrophils . High-affinity Lactoferrin receptors have been identified on several cell types including lymphocytes, monocytes, macrophages, enterocytes and platelets (Brock, 1995). Furthermore lactoferrin has been found to affect production of antibody synthesis, production of interleukin (I L)-1, I L-2 and TNF-α, natural killer cell cytotoxicity, complement activation and lymphocyte proliferation (Sanchez *et al*; 1992, Brock, 1995) .

Antimicrobial agents are, however still used to treat mastitis despite lack of evidence for their therapeutic value (Jones and Word 1990, Erskine *et al*, 1991; Pyorala *et al*, 1994). If exogenous administration of lactroferrin could support clearance of bacteria from the udder, then laetoferrin could be of value in the treatment of mastitis. Such efficacy of lactoferrin needs to be further investigated in the camel and other dairy animals .

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Table (4)

Mean (± SD) California mastitis test (CMT) score and NAGase activity in milk of endotoxin – treated camels

Time (hours)	CMT		NAGase (Units/ml)	
	C	E	C	E
0	1.01 ± 0.1	1.1 ± 0.1	12.1 ± 2.6	10.6 ± 2.1
3	1.1 ± 0.1	2.2 ± 0.2 *	11.2 ± 2.1	24.1 ± 2.4 *
6	1.1 ± 0.1	3.6 ± 0.3 *	11.6 ± 1.6	84.6 ± 4.6 *
24	1.1 ± 0.1	4.5 ± 0.4 *	10.1 ± 2.1	110 ± 6 *

C = control
E = endotoxin-treated
* P<0.001 Significantly different from control

Discussion:

Intramammary infusion of *E.coli* endotoxin in the udder of lactating camels has produced fever and slight swelling of the teats.

The increase in the concentration of the cellular component of milk of endotoxin treated animals such as polymorphnuclear cells indicated that endotoxin infusion into camel udder provoked leukocyte migration into milk. Similar pattern of response was observed in cows (Jain et al; 1978), goats (Lengemann and Pitzrick, 1987) and guinea pig (Mckenzie and Anderson, 1981) Furthermore, the increase in CMT score and NAGase activity indicated inflammatory reaction of udder to endotoxin infusion.

Lactoferrin in milk of endotoxin treated camels has significantly increased over the control camels reaching a level of 3.5 g/L at 24 hours post-infusion. Lactoferrin concentrations in milk from dairy cows with clinical mastitis has been reported to be 0.3-2.3 g/L (Harmon *et al* 1975, Kawai *et al* 1999). Such levels might be due to the severity of infection since in subclinical mastitis lower levels 0.2-1.2g/L were reported (Kawai *et al* 1999). Lactoferrin has been reported to be secreted by neutrophiles (Harmon and Newbould, 1980). It has been shown to be bacteriostatic for a variety of microorganisms in vitro as well as in vivo (Oram & Reiter. 1968: Reiter. 1985: Zagulski *et al.,* 1989: Bhimani *et al*., 1999). The inhibitory activity is believed to result from the iron chelating ability of lactoferrin making iron unavailable to bacteria (Weinberg. 1984). In addition, specific portions of lactoferrin molecules are directly toxic to

Table (1)

Mean (± SD) Percentage of leukocytes in milk of endotoxin or saline treated camels

Time (hours)	PMNC %		MNC %		CF %	
	C	E	C	E	C	E
0	2.2 ± 0.2	2.1 ± 2.2	55. ± 3	57 ± 4	43.3. ± 2.1	41.2 ± 2.1
3	2.3 ± 0.2	21.6 ± 1.2*	60.2 ± 2.1	62.1 ± 2.3	37.5 ± 2.1	17.3 ± 0.6*
6	4.4 ± 0.3	68.3 ± 2.6*	26.1 ± 1.3	26.6 ± 1.4	69.5 ± 2.1	5.1 ± 0.2*
24	4.3 ± 0.2	78.3 ± 3.3*	30.2 ± 1.2	20.3 ± 1.3*	65.5 ± 2.1	1.4 ± 0.1*

C = Control (Saline treated) E= Endotoxin treated PMNC= Polymorphnuclear cells ,
MNC= Mononuclear cells ,
CF=Cell fragments ,
*P<0.05 significantly different from controls.

Table (2)

Mean (± SD) differential leukocyte counts in endotoxin treated camels.

Type of cells% Total	Time hours			
	0	3	6	24
WBC ($x10^a$/L)	22 ± 1	15.4 ± 1.2	13.4 ± 1.3	16.1 ± 0.2
Lymphocyte	20.1 ± 1.1	24.2 ± 1.6	25.4 ± 1.6	10.4 ± 0.6
Neutiophils	65.1 ± 2.1	64.9 ± 1.9	71.2 ± 2.1	80.1 ± 2.2
Eosinophils	6.8 ± 0.4	4.3	3.4	4.1 ± 0.6
Basophils	4.6 ± 0.3	3.2 ± 0.2	2.1	2.6 ± 0.8
Monocytes	3.4 ± 0.4	3.4 ± 0.3	2.3	2.8 ± 0.4

*P<0.05 , Significantly different from Control**s**

Table (3)

Mean (± SD) Lactoferrin concentrations in milk of endotoxin or control saline-treated camels

Time (hours)	Lactoferrin (g/L)	
	Control	Endotoxin treated
0	0.12 ± 0.05	0.13 ± 0.04
3	0.15 ± 0.04	0.9 ± 0.11 *
6	0.14 ± 0.03	2.1 ± 0.12 *
24	0.12 ± 0.04	3.5 ± 0.21 *

* P<0.05 , Significantly different from Controls .

Lactoferrin determination in milk :

Lactoferrin concentration in milk samples was determined using dissociation-enhanced lanthanide fluoroimmunoassay (Kawai *et al*., 1999) as a comparative reaction and measured in a fluorometry (Biowhiltakar, MD, USA)

Statistical analysis:

Values were compared using student test. The probability value $P<0.05$ was considered significant .

Results :

The maximum increase in the rectal temperature of camels infused with endotoxin attained was 2.3^{o}C above control animals at 4 h post-infusion. No major pathogenic organisms were found in milk. Slight swelling of the teats was observed in endotoxin-treated animals .

A significant ($P<0.05$) increase in polymorph nuclear lenkocytes was observed in milk of endotoxin-treated camels compared to control camels (Table 1). Blood neutrophils were significantly ($P<0.05$) increased in endotoxin-treated camels (Table 2). The other cell types of the leukocytes series did not show any significant impressive change .

The milk NAGase activity has significantly ($P<0.05$) increased after endotoxin infusion . The highest value attained being 110 units/ml.

The CMT score has also significantly ($P<0.05$) increased 3 hours post infusion (Table 3).

The mean lactoferrin concentrations in milk is shown in (Table 4). Lactoferrin showed significant increase ($P<0.05$) at 3 hours post inoculation in endotoxin-treated camels and remained elevated reaching a level of 3.5 g/L at 24 hours post- inoculation . Basal levels were in the range of 0.12-0.15g/L .

dose of 10ug purified *E.coli* lipopolysacharide (055:B5, Sigma Chemicals UK) endotoxin in 3 ml of sterile isotonic saline solution (0.9% Nacl). The remaining five camels were used as control and the posterior quarters were infused with 3 ml of isotonic saline only. The udders were then massaged to ensure maximum distribution of endotoxin and saline .

Sample Collection : Oxytocin was administered to help milk let down. Quarter milk samples were collected before the endotoxin infusion (0 hour) and at post-infusion hours 3,6,22 and 24. Blood was obtained by jugular veinpuncture in vaccutainer tubes (Becton Dickson, Meulan, France) with sodium heparin as anticoagulant .

Clinical examination : The udder quarters were inspected and palpated and rectal temperature was taken at each sampling occasion .

Bacteriological examination : Milk samples (0.01 ml) from each quarter were streaked on blood agar plates and incubated for 48 hours at 37o C. The plates were examined for colony growth and morphology.

Milk somatic cell count : Quarter milk samples (0.01 ml) were spread over an area of l cm2 of a glass slide using a microsyringe. Four such squares were prepared form each sample. The smears were stained with methylene blue and examined under the microscope. Total cells/ml were counted according to Prescott and Breed (1990), and proportions of cells and cell fragments are estimated by counting 200 cells or particles .

Total and differential leukocyte count : The total blood leukocyte count was determined using Neubar haemocytometer after dilution . Blood was smeared on glass slides, air-dried and stained with Giemsa. And differential leukocyte count was made on 200 white blood cells .

California mastitis test (CMT) and milk N-acetyl-B-D-glucosaminidase (NAGase) activity determination:

The CMT and NAGse activity in milk were were determined and used as indicators of inflammation in the infused udder. The NAGase activity was analyzed using commercial kit (Roche Products, Herts, UK) based on the fluorgenic method of Kitchen *et al* (1978).

U.K.) at a dose of 0.1 µg/kg body weight to calves and adult camels induced fever and increased haematocrit, triiodothyronine and cortisol values. The endotoxin – treated animals showed significantly decreased ($P<0.05$) total protein, urea, glucose and creatinine. A significant increase was seen in the activity of aspartate amino transaminase and creatinekinase . These results demonstrated a high sensitivity of camels to *E.coli* endotoxin.

Mastitis is the most costly infectious disease in modern dairy farms. In addition to the expenses for treatment and discarded milk , income is lost because inflammatory damage to the affected mammary gland may temporarily or permanently reduce milk production . Limiting inflammation in the affected mammary gland during a clinical episode of mastitis may also limit damage to the gland and preserve milk production.

Changes in the number and type of cells in milk occur during mastitis (Guidry *et al*., 1983). Characterization of cells and estimation of lactoferrin in milk is an important step in understanding the defensive mechanism and changes during physiological and pathological states of the udder of dairy animals. Lactoferrin an iron-binding glycoprotein in milk, serves as part of the defence system (Baggioloni *et al*., 1970) Milk from clinically healthy bacterian camels contain few cells and numerous cell-like structures characterized by complete absence of nuclei but with intact cytoplasmic organelles (Abdurahman *et al*., 1992.)

The objective of this study was to investigate the effect of *E. coli* endotoxin infused into the camel udder and monitor the pattern of changes in lactoferrin concentration and cell populations in milk and blood .

Materials and Methods :

Animals : Ten clinically healthy female camels aged 6-7 years were used. Animals were fed on barley and wheat straw and water ad libitum The camels were in their 3-4 month of lactation and the calves were separated from their dams. The camels were sedated with xylazine (Img/kg; Rumpun, Bayer, Germany) before endotoxin infusion.

Endotoxin infusion : Five camels were used for endotoxin infusion. Two posterior quarters of the udder were infused through the teat canal with a

Effects of Intramammary Infusion of *Escherichia coli* Endotoxin in Lactating She-camel Udder

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Abstract :

Intramammary infusion of 10ug *E. Coli* lipopolysacharide endotoxin to lactating camels has increased blood neutrophils and milk polymorphnurclear cells, California mastitis test score and N-Cetyl-B-D-glucosaminidase activity. The infusion of the endotoxin had also increased the level of lactoferrin in milk . It is suggested that administration of lactoferrin could be of value in the treatment of mastitis.

Keywords :

Camel, endotoxin, *E. coli* , udder, lactoferrin, polymorphnuclear cells .

Introduction

Recent experimental findings indicate that endotoxin interacts with specific membrane receptors localized on mononuclear phagocytic cells and neutrophils (Olson *et al* 1995). Binding of endotoxin to these cells together with endotoxin – induced activation of host vascular endothelium, initiates a series of signal transudation events that culminate in relase of numerous biochemical mediators (Retz 1993). Endotoxaemia and Gram – negative septicaemia are important clinical entities in animals (Brigham and Meyrick, 1986; Ziegler *et al* (1991).

Indeed, introduction of endotoxin into the circulation appears to be a key factor initiating the pathophysiology associated with clinical shock during experimental endotoxaemia (Morrison and Ryan, 1987; Naess *et al*. 1989). There are marked species differences both in the sensitivity of animals to endotoxin and the dose of endotoxin required to achieve 100% lethality (Schrauwen and Houvenaghel 1985). Recently, it was demonstrated (AL-Dughaym, 2004) that intravenous administration of endotoxin prepared form *E. coli* (serotype 055:B5, Sigma Chemicals,

التغيرات الهرمونية المصاحبة للتبويض المتعدد المستخدم فيه الهرمون المنشط للغدة النخامية في الإبل

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الملخص :

تم أجراء الدراسة الحالية على ٧ نوق حقنت ٢٠ ميكروجرام من الهرمون المنشط للغدة النخامية في اليوم الأول للتجربه ثم تم فحص المبايض بجهاز الموجات فوق الصوتية لمتابعة نشاطها. بدأ علاج النوق لإحداث التبويض المتعدد عند أقل نشاط للمبايض. تكون علاج إحداث التبويض المتعدد من هرمونى الفرس المشيمائي المحفز للمناسل(جرعه واحده) والغدة النخامية المنشط للجريبات (جرعتين يومياً لمدة ٤ أيام). تم تلقيح النوق مرتين بينهما ١٢ ساعة باستخدام فحول خصبه عند وصول الجريبات حجم ١٣- ١٩مم. وقد استجابت كل النوق للتبويض المتعدد. وقد كان أقل عدد للأجسام الصفراء ٧ في حين كان اكبر عدد ٢٠ بمتوسط ١٣,٤٣ ±١,٧٧ جسم اصفر لكل ناقة. وقد تم قياس تركيز هرموني البروجستيرون والأستروجين خلال التبويض المتعدد.وبينت النتائج وجود ارتباط معنوي قوى سالب بين التبويض المتعدد و تركيز هرمون الأستروجين عند البدأ في إحداث العلاج للتبويض المتعدد فى حين لم يكن هناك ارتباط بين التبويض المتعدد و تركيز هرمون البروجستيرون فى نفس الفترة. كما تبين حدوث ارتباط معنوي بين التبويض المتعدد و تركيز هرمون البروجستيرون عند تقييم الاستجابة للتبويض المتعدد. وقد خلصت الدراسة أن هذا النظام للتبويض المتعدد قد أعطى نتائج مشجعة وأن السر في ذلك يكمن في العلاج بالمزج بين هرموني الفرس المشيمائي المحفز للمناسل والغدة النخامية المنشط للجريبات على أن يبدأ العلاج عند الغياب التام لجريبات المبيض، ويمكن التأكد من ذلك بالفحص بجهاز الموجات فوق الصوتية وقياس تركيز هرمون الأستروجين في الدم.

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combination of eCG and FSH as a superovulatory agent injected when there is no follicular activity. Absence of follicular must be confirmed by ovarian scanning and plasms estradiol assay.

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which was in the range of 2.5-4.5 ng/ml that reported in ovulated camels (Skidmore *et al.*,1997). However, the sharp increase of progesterone at day of recovery is probably associated with the increased number of corpora lutea. It was found that the low levels of progesterone at the day of recovery indicated premature regression of corpora lutea (Jensen *et al.*,1982; Lindsell *et al*.,1986).

The highly significant ($P<0.01$) negative correlation between the ovarian response of the experimental camels and plasma estradiol concentration at the start of superovulation coincided with the results of ovarian scanning at such time which revealed absence of follicular activity. Skidmore(2000) reported that if follicles are present at the time of treatment, these tend to develop into overlarge follicles before the new stimulated follicles have had chance to develop. On the other hand, the relationship between ovarian response of the experimental camels and plasma progesterone concentration at the start of superovulation was non-significant. However, in many experimental camels a higher ovarian response was obtained when progesterone concentration at the start of superovulation was low. On the contrary to this observation Goto *et al*. (1987) and Ismail *et al*. (1992a) reported a significant relationship between ovarian response and progesterone concentration at the start of superovulation in cattle. Callesen *et al*. (1988) informed that higher levels of progesterone at start of superovulation in cattle tended to suppress the basal luteinizing hormone (LH) discharge from initial injection of gonadotrophin to injection of prostaglandin; this allowed for greater storage of LH and subsequently produced a broader and higher LH surge resulting in higher ovulation response. However, in camels it seemes that low progesterone level at start of superovulation may stimulates the release of gonadotrophins for higher follicle formation. Then, LH surge is released from pituitary gland 3-4 hours after mating and ovulation occurred 24 to 36 hours later (Marie and Anouassi,1987). Higher LH surge was provided by mating the donor camels twice, 12 hours apart, with injection of GnRH at the first mating.

In conclusion, the current regimen of camel superovulaton produced a promising superovulatory response. The key for such result is the use of a

The response of all experimental camels (100%) to the superovulatory treatment and the high superovulatory response in the present work are considered promising results. High incidence of non-responsive camels to superovulation (developed less than 3Corpora lutea) was a problem reported by many investigators. Skidmore (2000) estimated an incidence of 20-30% non-responsive camels. The author attributed this result to immunization of some females against the superovulatory hormone. The high incidence of non-responsive camels was also noted by Cooper *et al.* (1992); Mc Kinnon and Tinson (1992); Vyas (1998); Ismail *et al.* (2006). Moreover, it seemed that this high incidence did not influenced by the physiological status of the camels whatever they were dry or lactating (Ismail and Al-Eknah, 2006) .

The superovulatory response of the experimental camels averaged 13.43±1.77 CL with a range of 7.00 to 20.00 CL.This ovarian response is much higher than the means of 4.6 to 5.7 CL reported by several authors (Skidmore *et al.*,1992; Ismail *et al.*,1993; Vyas,1998) injected the superovulatory hormone after 7 days progesterone priming via PRID or CIDR. The mean number of Corpora lutea obtained herein, is also higher than the means of 8.75 to 9.83 CL developed when the superovulatory hormone injected at the end of 10-15 days progesterone treatment (Mc Kinnon et al.,1994; Tinson et al.,2000; Ismail et al.,2006; Ismail and Al-Eknah,2006) . However, the high incidence of responsive camels to superovulation and the high ovarian response in this study are probably related to the regimen of superovulation, which guaranteed absence of follicular activity at treatment, and the use of combined eCG and FSH in the superovulation process. In this aspect, Skidmore (2000) stated that the best ovarian response in camels was seen when a combination of both eCG and FSH was used in the ovarian stimulation.

The increase of mean plasma progesterone concentration of the experimental camels from 2.21±1.01ng/ml before GnRH treatment to 4.33 ± 0.96ng/ml at superovulatory treatment coincided with the decrease of plasma estradiol concentration at the same periods. This may indicates the changing of the follicular structures into luteal structures as a result of GnRH treatment. This is justified by the level of plasma progesterone

Table (3)

Plasma progesterone and estradiol 17ß concentrations during the different phases of the superovulatory regimen in the experimental camels (Mean ±SEM)

Time of plasma sampling	Progesterone Concentrations (ng/ml)	Estradiol 17ß Concentrations (pg/ml)
Just before GnRH treatment	2.21±1.01	22.00±1.03
At superovulatory treatment	4.33±0.96	18.75±1.84
At day of recovery	18.2±4.78	22.09±1.21

Table (4)

Correlation coefficients (r) between ovarian response and Plasma progesterone and estradiol concentrations during the different phases of the superovulatory regimen of the experimental camels (Mean ±SEM).

Ovarian response	Progesterone concentration (ng/ml)	Estradiol 17ß concentration (pg/ml)
Just before initiation of the superovulatory treatment	-0.60	-0.84**
At the day of recovery	0.73*	-0.23

**Significant at P<0.01 *Significant at P<0.05

successful superovulation is the absence of ovarian follicles at the start of superovulatory treatment. This concept was supported in the current study by the significant negative correlation (P<0.01) between the ovarian response and the plasma estradiol concentration at start of the superovulatory treatment. Similar findings were reported by Tibary and Anouassi (1997); Skidmore (2000); Ismail *et al*.,(2006); Ismail and Al-Eknah (2006) who found that the best stimulation of the camel ovaries occurred when treatment with exogenous gonadotrophic hormone started at minimum follicular activity. The presence of follicular activities at the time of treatment is probably associated with high estrogen level which blocked the release of pituitary FSH and cause low ovarian response (Saumande, 1980)

and the plasma estradiol (r= -0.84) concentration. However, the correlation between the ovarian response and plasma progesterone concentration was non-significant (r= -0.60) .At the day of recovery the correlation coefficient between ovarian response and the plasma progesterone concentration (r= 0.73) was significant (P<0.05). However, the correlation coefficient between ovarian response and the plasma estradiol concentration (-0.23) was non-significant .

Discussion:

The regimen of superovulation used in the current study based upon priming the donor camels with GnRH followed by daily scanning of the ovaries for six successive days. On day seven postGnRH treatment, when there was no follicular activity a combination of eCG and FSH-P was injected. It was planned to overcome any follicular activity present before initiation of the

superovulation by daily treatment with 100 mg progesterone in sesame oil until disappearance of the follicular structure.However, all experimental camels had no follicular structyres at commencement of superovulation. It is evident that the key for

Table (2)

The ovarian response of the experimental camels (Mean ±SEM)

Animal Number	Number of corpora lutea (Corpora lutea)			Number of follicles		
	L	R	Total	L	R	Total
5	6	3	9	-	2	2
6	8	10	18	1	-	1
2	4	3	7	1	1	2
3	6	3	9	1	1	2
1	5	10	15	-	-	-
4	4	11	16	-	2	2
7	6	13	20	-	2	2
mean	1.57 ± 0.28			13.43 ± 1.77		

measuring both hormones in unknown samples. The sensitivity of the assay defined as the smallest concentration significantly ($P<0.05$) distinguishable from zero was 0.1ng/ml and 3pg/ml plasmas for progesterone and estrogen hormones, respectively. The intra-and inter assay coefficients of variation were 7.9 and 8.5% for progesterone and 12.3 and 16.8% for estrogen hormones, respectively.

6. Statistical analysis

Data were expressed as mean ± SEM. Correlation coefficient was used to correlate between the ovarian response and both of plasma progesterone and estradiol concentrations. Statistical analysis was performed according to Snedecor and Cochran (1979).

Results:

1. Superovulatory response

All experimental camels (100%) responded to the current regimen of superovulation The lowest response (7Corpora lutea) was observed in camel number 2 while the highest response (20Corpora lutea) was recorded in camel number 7 (Table2).The over all average of the number of ovarian corpora lutea was estimated to be 13.43±1.77.The number of anovulated follicles ranged from 0 to 2 with an average of 1.57±0.28 (Table 2)**.**The ovulation rate was estimated to be 89.52%.

2. Hormonal analysis

Plasma progesterone concentrations showed an increase from 2.21 ± 1.01 ng/ml before GnRH injection to 4.33 ± 0.96 ng/ml at the time of superovulatory treatment. A sharp increase in plasma progesterone concentration to 18.2 ± 4.78 ng/ml was measured 8 days post-mating. Plasma estradiol 17ß concentration was nearly constant throughout the experimental period. It fluctuated around 22.00 pg/ml before GnRH treatment and 18.00 pg/ml 8 days post-mating (Table, 3).

3.Hormonal-ovarian response relationship

Correlation coefficients between the ovarian response and both of plasma progesterone and estradiol concentrations are shown in table (4). Just before initiation of of the superovulatory treatment, a highly significant negative correlations ($P<0.01$) was observed between the ovarian response

of terminating the superovulatory hormone until the majority of follicles were considered sufficiently mature (1.3 - 1.9 cm in diameter), where mating with fertile male camels was allowed twice 12 hours interval. Each female camel received 20 µg buserlin just at the time of first mating.

Table (1)
Regimen of superovulation in the experimental camels

Day of experiment	Event
1	Injection of 20 µg buserlin
7 (With minimal ovarian activity)	Am 2000IU eCG + 4ml FSH Pm 4ml FSH
8	Am 3ml FSH Pm 3ml FSH
9	Am 2ml FSH Pm 2ml FSH
10	Am 1ml FSH Pm 1ml FSH
14	Mating + Injection of 20µg buserlin
22	Evaluation of the ovarian response

4. Evaluation of the ovarian response

The ovarian response of the experimental camels was evaluated 8 days post-mating (7 days post-ovulation). Evaluation was conducted by rectal palpation and ultrasonography. Two camels were subjected to laparotomy for more accurate counting of the excessive number of corpora lutea.

5. Hormonal assay

Blood samples were collected into heparinized tubes by jugular venipuncture just before GnRH treatment throughout until the time of mating. Plasma samples were stored at -20°C until hormonal analysis. Plasma estradiol -17ß was measured according to the method adopted by Landgren et al.(1982) whereas plasma progesterone was measure using the method employed by Sheehan *et al*. (1982). Both hormones were measured using Coat-A-count kits (Diagnostic Products Corporation,USA). Gamma counter (Berthold) was used for counting and the produced number was converted by the way of calibration curve for

perhaps, would contribute to substantial savings of financial and time resources. In cattle and buffaloes, several studies (Landgren *et al*.,1982; Goto *et al*.,1988; Ismail *et al*.,1992a,b) have shown an intimate relationship between plasma progesterone and estradiol levels at start of superovulation and the superovulation response. Moreover, ovulation rate, embryo production and embryo quality can be predicted by the plasma levels of these hormones (Saumande *et al*.,1985; Tamboura *et al*.,1985; Goto *et al*.,1987). In camels similar information are scanty.

The present research aims to explore a different regimen for camel superovulation. In addition, plasma progesterone and estradiol concentrations are measured during different periods of the regimen. The relationship between the plasma concentration of these hormones and the superovulatory response is considered.

Materials and Methods:

1. Camels

Seven mature non-pregnant female camels (*Camelus dromedarius)* were used in the present study. They were 6 to 14 years old, kept in open yard and fed on barley and rhodes grass hay. Water was provided ad libitum. Camels were kept in the Camel Research Centre, King Faisal University , during the experimental period.

2. Priming and Superovulation

Experimental camels were examined by ultrasonography before commencement of any injection .All showed follicular activities of different sizes. All camels were injected with 20 µg. buserlin (Receptal^R, Intrervet Ltd., Holland) at the first day of the experiment. 7 days later, the camels were scanned for ovarian activities. Superovulatory treatment started when a minimal ovarian activity was observed. Superovulatory treatment consisted of a combination of FSH (FSH-P^R,Sigma, USA) and eCG (Folligon^R, Intrervet Ltd., Holland) hormones. Regimen of superovulation is presented in Table1.

3. Mating and ovulation

The development of ovarian follicles was monitored by ultrasound scanner. Scanning was daily performed for all camels starting the next day

In conclusion, the current regimen of camel superovulaton produced a promising superovulatory response. The key for such result is the use of a combination of eCG and FSH as a superovulatory agent injected when there is no follicular activity. Absence of follicular activity must be confirmed by ovarian scanning and plasma estradiol assay.

Introduction:

The opportunities of improving camel reproductive efficiency are limited due to the continued use of traditional systems of reproductive management in most breeding herds (Cooper *et al*.,1990). The use of embryo transfer (ET) technique in the camel breeding industry can be of a particular value to increase the number of progenos from desirable male and female genetic combinations, whether this be for racing or production of meat or milk (Yagil and Van Creveled,1990). Moreover, ET could provide more progeny from subfertile camels and those calving late in the breeding season. It could also be used to test techniques such as artificial insemination with fresh, cooled or frozen semen (Mc Kinnon *et al*.,1994).

The fundamental objective of superovulation is to increase the number of fertile eggs given by the treated outstanding female. Optimum superovulatory response in farm animals achieved when the superovulatory hormone was administered during the midluteal phase of the cycle which was subsequently curtailed one to three days later by prostaglandin hormone (Lerner *et al*.,1986; Ismail, 1991). However, superovulation is a challenge in camels as they are induced ovulators and corpus luteum is only developed when mating occurs (Al-Eknah,2001). Superovulation in the camel, therefore, was obtained by injecting eCG or FSH hormones at the end of induced luteal stage which provided by progesterone releasing intravaginal device (PRID), controlled intravaginal device release (CIDR) or daily progesterone injection (Anouassi and Ali,1990; Skidmore et al.,1992; Ismail et al.,1993; Mc Kinnon *et al*.,1994; Tinson et al.,2000; Ismail et al.,2006; Ismail and Al-Eknah,2006).However, attempts of superovulation in the camel have generally yielded poor and inconclusive results (Purohit,1999)

It is of great interest in embryo transfer programs, prior to induction of superovulation, to predict the non- or poor responsive donors. This,

Hormonal Changes During Busserlin (GnRH) Priming Regimen for Superovulation in the Camel (*Camelus dromedarius)*

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Abstract:

Seven mature non-pregnant female camels (*Camelus dromedarius)* have been used in this study. The female camels were injected with 20 µg. buserlin at the first day of the experiment, then scanned for ovarian activities. Superovulatory treatment commenced when a minimal ovarian activity was observed. Superovulatory treatment consisted of a combination of follicle stimulating hormone (FSH) and equine chorionic gonadotrophin (eCG) . One dose of 2000IU eCG was intramuscularly injected for all female camels, but 50mg FSH was splitted and injected twice daily for 4 consecutive days in a decreasing manner. The female camels were twice mated (12 hours apart) with fertile male camels when the ovarian follicles are considered sufficiently mature at 1.3 to 1.9 cm in diameter. Each female camel received 20 µg buserlin at the time of the first mating. All experimental camels responded to the superovulation regimen. The lowest response was 7Corpora lutea, while the highest response was 20Corpora lutea. The over all average of the number of ovarian corpora lutea was 13.43±1.77. The number of anovulated follicles ranged from 0 to 2 with an average of 1.57±0.28. Plasma progesterone concentrations showed an increase from 2.21 ± 1.01 ng/ml before GnRH injection to 4.33 ± 0.96 ng/ml at the time of superovulatory treatment. A sharp increase in plasma progesterone concentration to 18.2 ± 4.78 ng/ml was measured 8 days post-mating. Plasma estradiol 17 ß concentration was nearly constant throughout the experimental period. It fluctuated around 22.00 pg/ml before GnRH treatment and 18.00 pg/ml 8 days post-mating. A highly significant negative correlations ($P<0.01$) was observed between the ovarian response and the plasma estradiol ($r= -0.84$) concentration before initiation of superovulation. There was a significant ($P<0.05$) correlation coefficient between ovarian response and the plasma progesterone concentrations ($r= 0.73$) at the day of recovery.

بقايا المبيدات الكلورنية العضوية في دهون الدجاج اللاحم في أسواق المنطقة الشرقية – المملكة العربية السعودية

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الملخص:

لقد تم مسح بقايا المركبات الكلورنية العضوية في دهون الدجاج اللاحم في الأسواق المركزية في المنطقة الشرقية بالمملكة العربية السعودية وكذلك في مزارع الدجاج اللاحم والنتافات بالأحساء خلال شهري مايو ويونيو ٢٠٠٥ م وقد أوضحت الدراسة أن العينات التي جمعت من أسواق القطيف والدمام والأحساء قد احتوت على بقايا مركب الاندوسولفان ومركب الهيباتوكلور ولكن بتركيز يقل كثيرا عن الحد الأقصى المسموح به في دهون الدجاج حسب التشريعات الأوربية (EEC/DC 1986). كما خلصت الدراسة إلى ضرورة الاستمرار في إجراء مسح دوري مستمر للكشف عن هذه المركبات حتى يمكن تفادي بقايا المبيدات الحشرية في لحوم الدجاج حفاظا على صحة المستهلك.

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Table (2) Organochlorine residues[a] in chicken fat collected from market of Eastern Region of Saudi Arabia

Source	Number of samples	Positive samples			
		Hepatochlor (MRL[b]= 200 µg/Kg)	Range (µg/Kg)	Endosulfan (MRL= 200 µg/Kg)	Range (µg/Kg)
Jubail	25	-	-	-	-
Qatif	20	-	-	1	20
Dammam	25	-	-	1	15
Khobar	20	-	-	-	-
Abqaiq	20	-	-	-	-
Al-Ahsa supermarket	30	-	-	2	10-20
Al-Ahsa poultry farms	100	1	15	2	10-20
Al-Ahsa chicken pluck shops (Natafa)	100	3	10-20	3	10-20

a) Other organochlorine compounds were not detected.

b) MRL= Maximum residue limit.

Results and Discussion:

Residues of organochlorine compounds in chicken fat collected from supermarkets, poultry farms and chicken pluck shops (Natafa) of Eastern Region are shown in Table 2. Few samples of chicken fats collected from Qatif, Dammam and Al-Ahsa were found to contain residues of endosulfan and hepatochlor.

The residues detected were well below the maximum residue limits for fat tissues according to European regulations (EEC/CD 1986). Occurrence of pesticides and herbicides in soil and sand dust from Riyadh city has been confirmed (Al-Mutlaq *et al*., 2002; Rushdi *et al*., 2004). Local agriculture and gardening application of these pesticides may be the main source of compounds in the dust of city of Riyadh and elsewhere in the Kingdom. Uses of these pesticides and insecticides in poultry premises, however, could not be ruled out. Evidence of organochlorine pesticides contaminating poultry tissue in different parts of the world has been reported (Watson *et al*., 1993; Doganoc, 1999; Cerkvenik and Komar, 1999; Jevsrick *et al*., 2004). In this study, the sample size was rather small. A wider scale study including survey of eggs may yield more informative data. It is note worthy to conclude that endosulfan was detected in major cities of Eastern Region of Saudi Arabia. Endosulfan is registered as hazardious materials causing acute and chronic toxicities (Khare *et al*., 2002) among which is reduction of oxygen uptake in blood (Bhalchandra and Lemote, 2003).

In conclusion, the presence of hazardous pollutants in chicken fat is mainly due to environmental contamination. This needs continuous close monitoring and corrective action.

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After dissection, chicken fat tissues were packed separately in plastic bags. Fat tissues was then homogenized and melted at 80°C. An aliquot was stored in a fridge at 4°C until analysis.

Analytical method:

Samples of fat were extracted and assayed according to the method of Frank *et al*. (1985). The compounds detected were hexachlorocyclohexane (HCH), endosulfan, lindane, hepatochlor, methoxychlor, dieldrin, endrin and total DDT.

Insecticides residues were measured by electron capture (^{63}Ni source) Gas chromatography (Hitachi, Japan) using a 15 m x 0.25 mm capillary column coated with an 0.25 µm thickness of SE-30. Chromatographic conditions were as follows: injector temperature, 225°C; detector temperature, 300°C; column oven, programmed for an initial 1-min hold at 90°C followed by 15°C/min to 20°C and then 5°C/min to 25°C; carrier gas, helium at 30 cm/s (105 kPā head pressure) and 30 ml argonmethane (95 + 5)/min make-up gas to the detector. The injection was auto injection of 1.5 µl with capillary inlet system configured in the splitless mode with the by-pass valve held open for 0.5 min.

Recoveries were determined by direct fortification of fat with known concentration of compounds in acetone and analyzed as described above. Results were corrected for extraction losses. Detection limits of the assay and the recovery values are shown in Table 1.

Table (1)

Average recoveries and detection limits in fat

Analyte	Recovery (%)	Detection limit (µg/kg)
Total DDT	87	0.4
HCH	88	0.1
Methoxychlor	81	1.0
Heptachlor epixode	91	0.1
Dieldrin	83	0.1
Endrin	80	0.2
Endosulfan	88	0.1
Lindane	94	0.1

Organochlorine Pesticidal Residues in Broiler Chicken Fat in Markets of Eastern Region of Saudi Arabia

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Abstract:

This survey was conducted to investigate the presence of organochlorine compounds in chicken fat collected from supermarkets of cities of Eastern Region of Saudi Arabia during May-June, 2005. Few samples of chicken fat collected from Qatif, Dammam and Al-Ahsa were found to contain residues of endosulfan and hepatochlor below the maximum residue limits for fat tissues. Close monitoring of residues is suggested to minimize ingestion of these compounds.

Introduction:

Organochlorine compounds are categorized by the Agency of Toxic Substances and Disease Registry (ATSDR, 1994) as probably carcinogenic to human. The presence of these pollutants in the environment (Fraser *et al*., 1998; Buehler *et al*., 2001) leads to their presence in foods (Frank et al., 1985). Biomonitoring procedures have been developed to assess human exposure to organochlorine and other pesticides to ensure that residues are kept at low level so that health risk posed by their ingestion is minimized. This survey was carried out to obtain information on the occurrence of organochlorine compounds in chicken meat collected from markets of Eastern Region of Saudi Arabia.

Materials and Methods:

Sampling Procedures:

Samples of frozen broiler chicken were collected from supermarkets in Jubail, Qatif, Dammam, Khobar, Abqaiq and Al-Ahsa in a single trip during May-June, 2005. Additional samples were collected from 5 broiler farms and 10 chicken pluck shops (Natafa) in Al-Ahsa region.

دراسة المتغيرات الدموية والحيوية في دم الأغنام بعد أطعامها عليقة منخفضة من نبتة عيينة (عين الجمل أو صابونة الغيط)

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الملخص :

في هذا البحث تم دراسة المتغيرات الدموية والحيوية في دم الأغنام بعد أطعامها عليقة تحتوي على ٥جم لكل كجم من الوزن الحي للحيوان من نبتة عيينة (عين الجمل أو صابونة الغيط). انزيم اية ال تي (ALT), الجلوكوز والكرياتينين اظهرت زيادة معنوية مقارنة بتركيز تلك المواد في الدم فبل إعطاء النبتة. اما كريات الدم الحمراء والبيضاء والهيموجلوبين وحجم الجزئيات المظغوطة أظهرت نقص معنوي مقارنة بتركيز تلك المواد قبل إعطاء النبتة. وكذلك أظهرت الدراسة عدم وجود تغيرات جوهرية في مستوى الكالسيوم والفوسفور والمغنيسيوم واليوريا وانزيم اي سي تي (AST) . لذا تخلص هذه الدراسة بان نبتة عيينة (عين الجمل أو صابونة الغيط) ذات تأثير سام في الأغنام بالجرعة المنخفضة (٥ جم /كجم من الوزن الحي يوميا/ ٤ أسابيع) التي استخدمت في هذه الدراسة.

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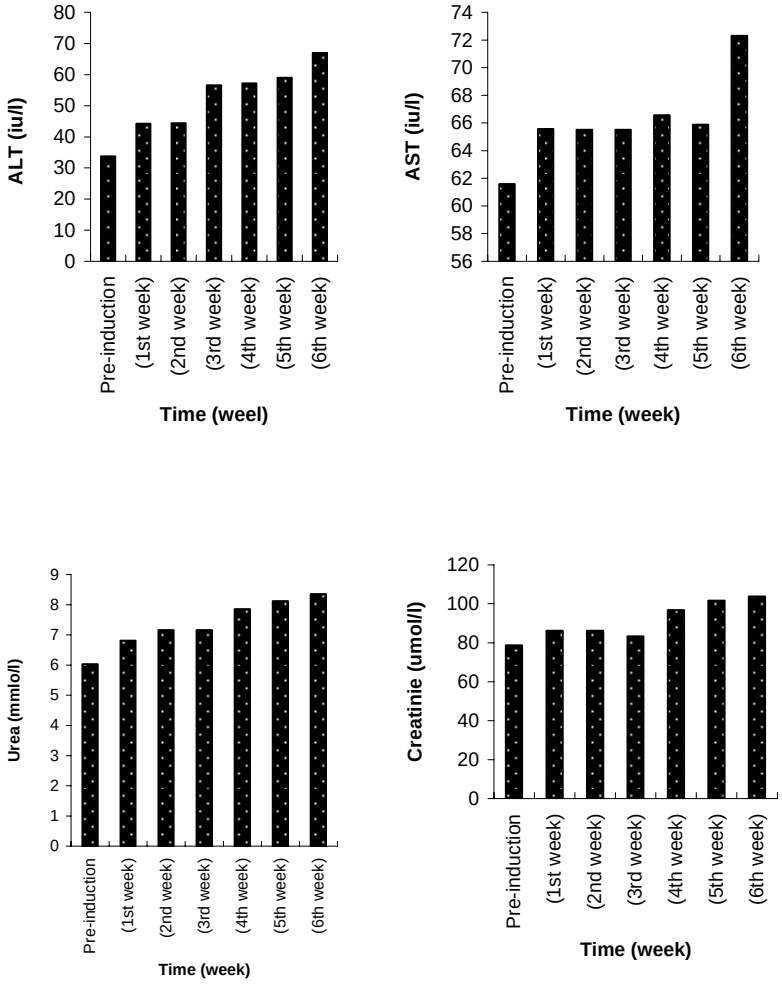

Fig. (3) : The concentrations of plasma urea, creatinine, ALT and AST in sheep blood prior after 5 weeks of feeding *Anagallis arvensis*

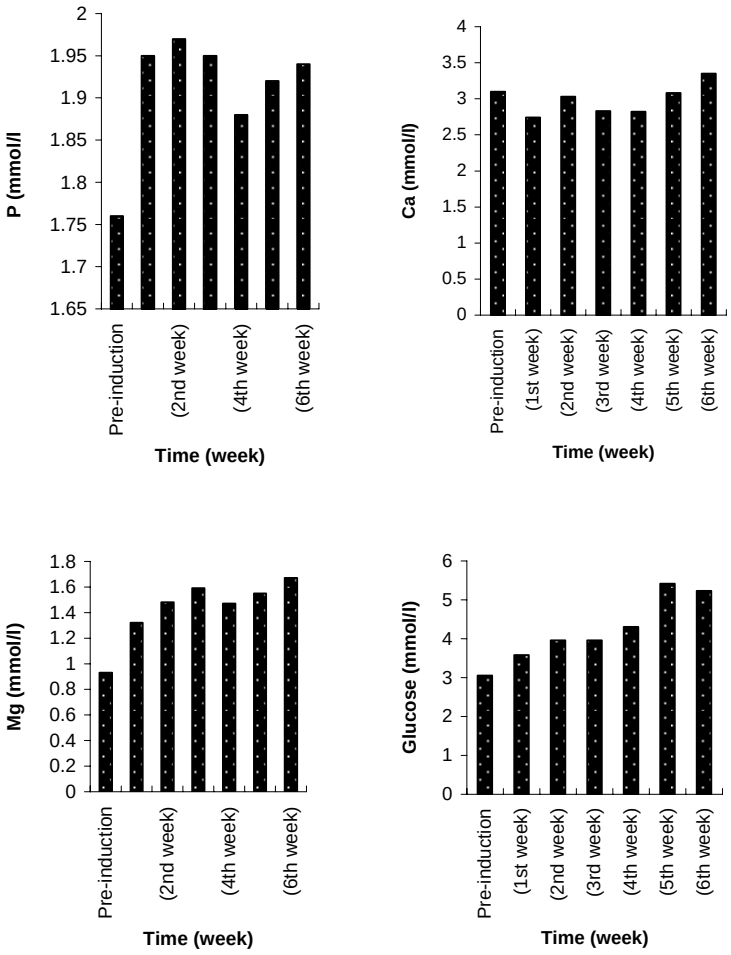

Fig. (2) : The concentrations of plasma glucose, calcium, magnesium and inorganic phosphate in sheep blood prior and after 5 weeks of feeding *Anagallis arvensis*

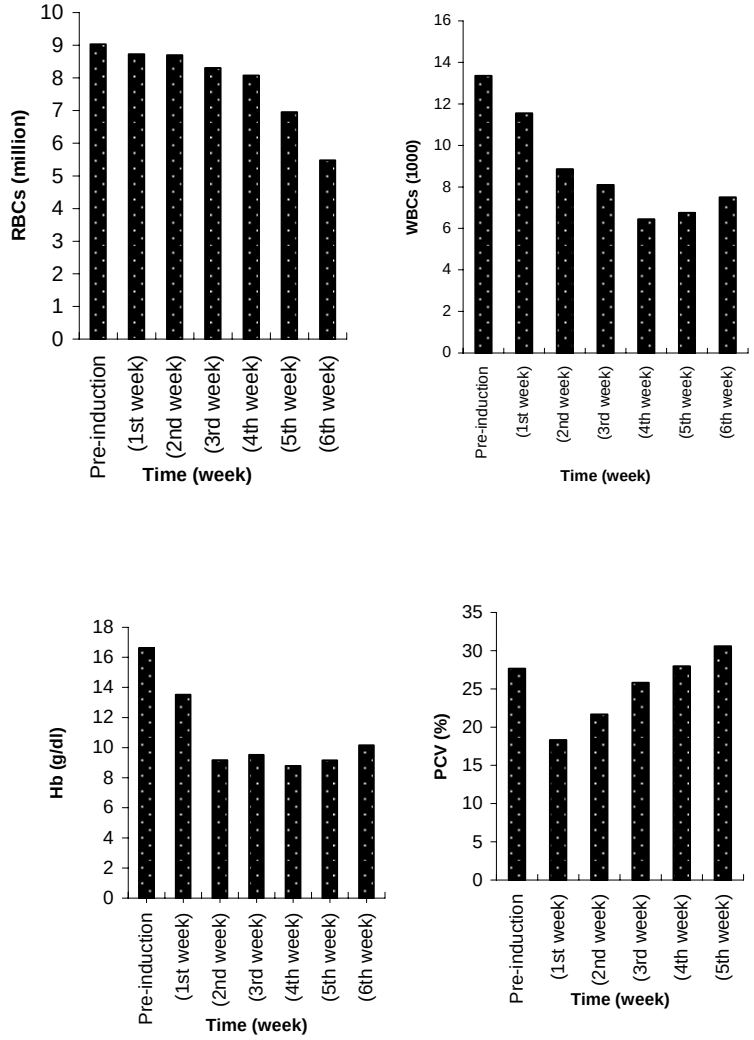

Fig. (1) : The concentrations of RBC, WBC, Hb, PCV in sheep blood prior and after 5 weeks of feeding *Anagallis arvensis*

statistical analysis : One way analysis of variance (Anova) was performed on the data and means were compared by the Duncan test.

Results & Discussion:

The results of the hematological and biochemical analysis are presented in Fig. 1, 2 and 3.

There was highly significant increase in ALT, glucose and creatinine levels. The hematological study revealed highly significant decrease ($p< 0.05$) in RBCs, Hb%, PCV and WBCs. There was no any significant changes of Ca, P, Mg, AST and urea.

Studies on the toxicity of *Anagallis arvensis* in sheep were done using small dose (5 g/kg.b.wt.) for 4 weeks. The clinical signs observed on sheep were mild and were mainly characterized by anorexia , restlessness, diarrhea, thirst and increased respiration. These signs were similar to those mentioned by Watt and Breyer – Brandwijk, (1962), Forsyth, (1968) and Kotb, (1985).

Hematological analysis revealed the occurrance of severe anemia as indicated by decreased hemoglobin concentration, lowering of packed cell volume and fall in erthrocytic count. These results could be attributed to the harmful effect of saponin, an active principle of *Anagallis arvensis*. ElGarieb (1990) reported similar findings.

The level of creatinine in the experimental sheep was increased significantly ($p< 0.05$). This increment is an indicator for renal function impairment due to toxicosis by *Anagallis arvensis* (Coles 1974, Metwalli 1987, and El.Garieb 1990). Increase in ALT is indicative of acute hepatic disease (Wrohlewski and La Due, 1956). There was a significant increase in serum glucose level of sheep. These results may be attributed to its role on hepatocites.

It could be concluded that *Anagallis arvensis* was toxic to sheep in low doses and induces several changes in blood picture and biochemical changes. This toxicity was reported previously in sheep by Riet-Correa *et al*. (1998) and Sedekar *et al*, (1996).

anorexia and constipation after feeding *Anagallis arvensis* with pathological changes in kidneys and livers in sheep.

When the green plant collected from a field where an outbreak had occurred was administered to sheep at doses of 160 and 224 g/kg bw clinical and pathologyical signs similar to those observed in field cases were observed (Rivero, *et al*., 2001).

Cases of acute mortality in sheep, characterised by severe nephrosis and resultant uraemia, were investigated on two farms 150 km apart in the Winter Rainfall area of the Republic of South Africa. This condition was experimentally reproduced by dosing sheep with *Anagallis arvensis* L plants. The most consipicious lesion was coagulative necrosis with intratubular haemorrhage in the renal cortex. The, clinical signs and the pathology of the experimental disease were described (Schneider, 1978).

The present study was undertaken to study the haematological and biochemical changes in sheep fed low dose of the plant *Anagallis arvensis*.

Materials and Methods:

Plants: *A.arvensis* plant was freshly collected every day from different farms in Al-Ahsa area.

Animals: six adult female sheep were used in this experiment. They were obtained from sheep farm of Training and Research Station, King Faisal University. The leaves of the: *Anagallis arvensis* plant was administered to the animals in a dose of 5 g./kg.bwt. daily for 4 weeks. Blood samples were taken every week for haematological and biochemical analysis. Also clinical signs were observed daily.

Blood picture: Total RBCs, WBCs, Hb, and PCV, were recorded by automatic coulter counter,.

Biochemical analysis included Creatinine, BUN, GPT(ALT),GOT(AST), and glucose. Also mineral(Ca, Mg and P) were analysed according to the set description of Bayer Corporation, SERA PAK (1997), with the aid of AMES QUIK-LAB chemistry analyzer, Miles Inc., Germany.

Haematological and Biochemical Changes in Sheep Associated with Low Dose Feeding of *Anagallis Arvensis*

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Abstract:

In this study the hematological and biochemical blood constituents of sheep fed low doses of *Anagallis arvensis* were investigated. The levels of alanine aminotransferase (ALT), glucose, and creatinin when compared with preinduction levels showed significant increase levels. On the other hand red blood cell (RBC), white blood cell (WBC), Hemoglobin (Hb) and packed cell volume (PCV) were significantly lower than preinduction values. However calcium (Ca), phosphorus (P), magnesium (Mg), aspartate aminotransferase (AST) and urea values were within the normal levels. The present findings indicated that *Anagallis arvensis* was toxic to sheep in the daily low dose of 5 gm/kgbwt .

Introduction:

The plant family primulaceae in Saudi Arabia cosists of 4 genera with 4-5 species (Shaudhary and Al-Jowaid, 1999). *Anagallis arvensis* L. is a polymorphic species widely spread in Al-Ahsa, where it grows well in wet habitat, heavy alluvial soil, canal banks and also as a common weed in cultivation.

Kotb,(1985) reported that the active principle of *Anagallis arvensis* were acired volatile oil, enzymes , saponins , tannins , bitter principle and a compound known as primin. Roots contain cyclamen, a crystallizable glucosidal saponin. Chevallier (1996), recorded that *Anagallis arvensis* contains saponins (including anagallin), tannins and cytotoxic substance. The plant was found toxic to dogs, rabbits and sheep,with signs of general depression , thirst and diarrhea (Riet-Correa *et al* 1998).The same authors described four cases of *Anagallis arvensis* poisoning in cattle of different ages. Morbidity rate was 7-30% and case fatality rate was 50-86%.. The animals were in the fields 7 to 45 days before developing clinical signs. Eight of 289 ewes died after grazing in the same field after showing clinical signs for 2-15 days. Sadekar *et al* (1996), reported dullness,

تحسين نمو نخيل التمر صنف خنيزي بإضافة دبس التمر لبيئة الإكثار النسيجي

عبداللطيف على الخطيب

مركز أبحاث النخيل والتمور، جامعة الملك فيصل

الأحساء ، المملكة العربية السعودية

الملخص:

أجرى البحث بمعمل زراعة الأنسجة بمركز أبحاث النخيل والتمور، جامعة الملك فيصل وذلك بغرض تحسين نمو الأنسجة في نخيل التمر صنف خنيزي عن طريق إضافة عدة تركيزات من دبس التمر وهي كالآتي ٠,٥ ، ١٠ ، ٢٠٪ بالإضافة إلى سكروز بمعدل ٣٠ جم/لتر معاملة (الكنترول). وأظهرت النتائج أن التركيزات المنخفضة من دبس التمر (٥٪) نشط تكوين البراعم بنجاح بينما التركيز الأعلى (١٠ و ٢٠ %) أدّى إلى التلون باللون البنى وجفاف الأجزاء النباتية. هذه الظاهرة محتمل أن سببها التأثيرات الأسموزية لهذين التركيزين العاليين من الدبس لذلك لا ينصح باستخدامهما. كما بينت هذه الدراسة أن تركيز ٥٪ دبس التمر قد حسن معظم الصفات الخضرية تحت الدراسة مثل النموات القابلة للتجذير والوزن الرطب والجاف للنموات الخضرية.

على ضوء هذه النتائج أجريت تجربه أخرى أستخدم فيها التركيزات التالية من دبس التمر ١, ٢, ٣, ٤, ٥ و ٦٪. بينت هذه الدراسة أن التراكيز ٤ و ٥ و ٦٪ أدت إلى تكوين البراعم و بدرجة كبيرة مقارنة لتركيز ٣٠جم/ل سكروز إن لم تكن متفوقة عليه, وصلت الى درجة المعنوية وخاصة تركيزي ٥ و ٦٪ دبس التمر, بينما التراكيز ١ و ٢ و ٣٪ أدت إلى قلة تكوين البراعم والنموات القابلة للتجذير بسبب قلة تركيز السكريات في هذه التراكيز. كما أوضحت النتائج مقدرة نموات النخيل على إستخدام دبس التمر كمصدر وحيد للكربوهيدرات بدلا من السكروز. يعتبر هذا هو التقرير الأول في إستخدام دبس التمر في عملية الإكثار النسيجي للنخيل. وأخيرا يمكن القول أن التراكيز ٤, ٥ , وكذلك ٦٪ من دبس التمر يمكن أن تحل كاملا مكان ٣٠جم/ل سكروز وهو التركيز المستخدم عادة في الإكثار النسيجي لنخيل التمر.

الكلمات الدالة: النخيل, الإكثار النسيجي, سكروز, دبس التمر

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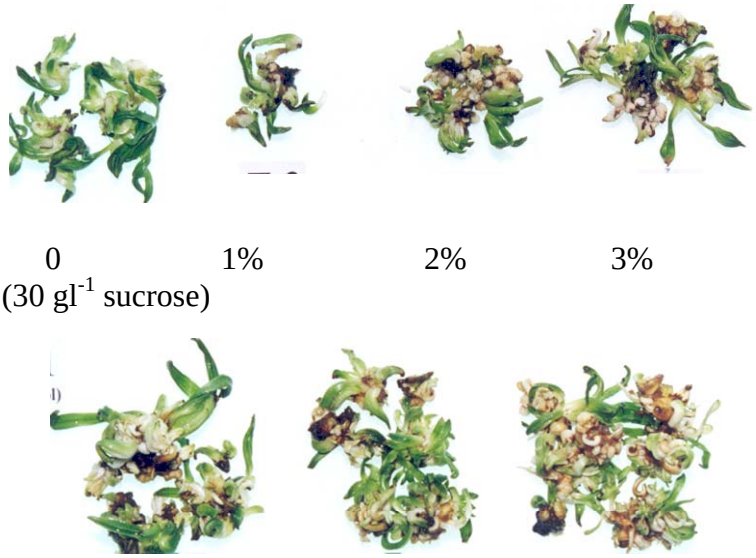
0 1% 2% 3% 4% 5% 6%
(30 gl^{-1} sucrose)

Fig. (6): Effect of date syrup (Dibs) on bud and shoot formation in flasks of Khanezi date palm cultivar (note color media degree) in the second experiment.



0 1% 2% 3%
(30 gl^{-1} sucrose)

4% 5% 6%

Fig. (7): Effect of date syrup (Dibs) on bud and shoot formation of Khanezi date palm cultivar in the second experiment.

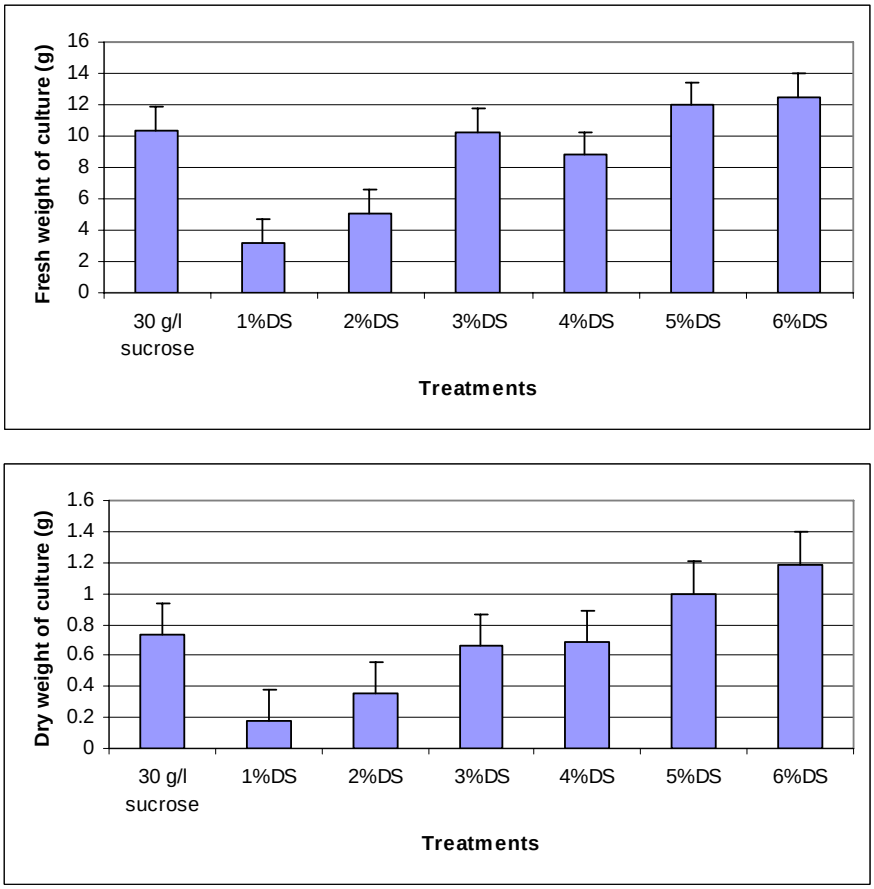


Fig (5): Fresh and dry weights of culture of date palm (*Phoenix dactylifera)* cv. Khanezi *in vitro* tissue culture as affected by different concentration of date syrup. Lines over bars represent LSD at 5%.

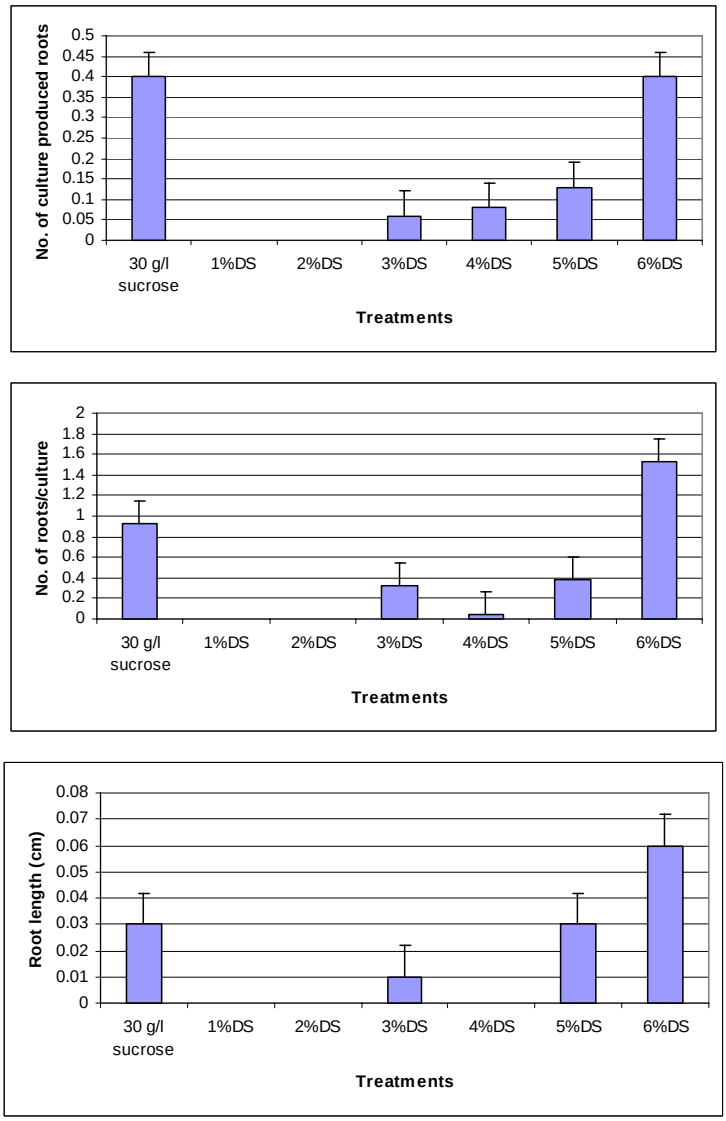


Fig (4) : Number of culture produced root, no. of roots/culture and longest of root length of date palm (*Phoenix dactylifera)* cv. Khanezi *in vitro* tissue culture as affected by different concentration of date syrup. Lines over bars represent LSD at 5%.

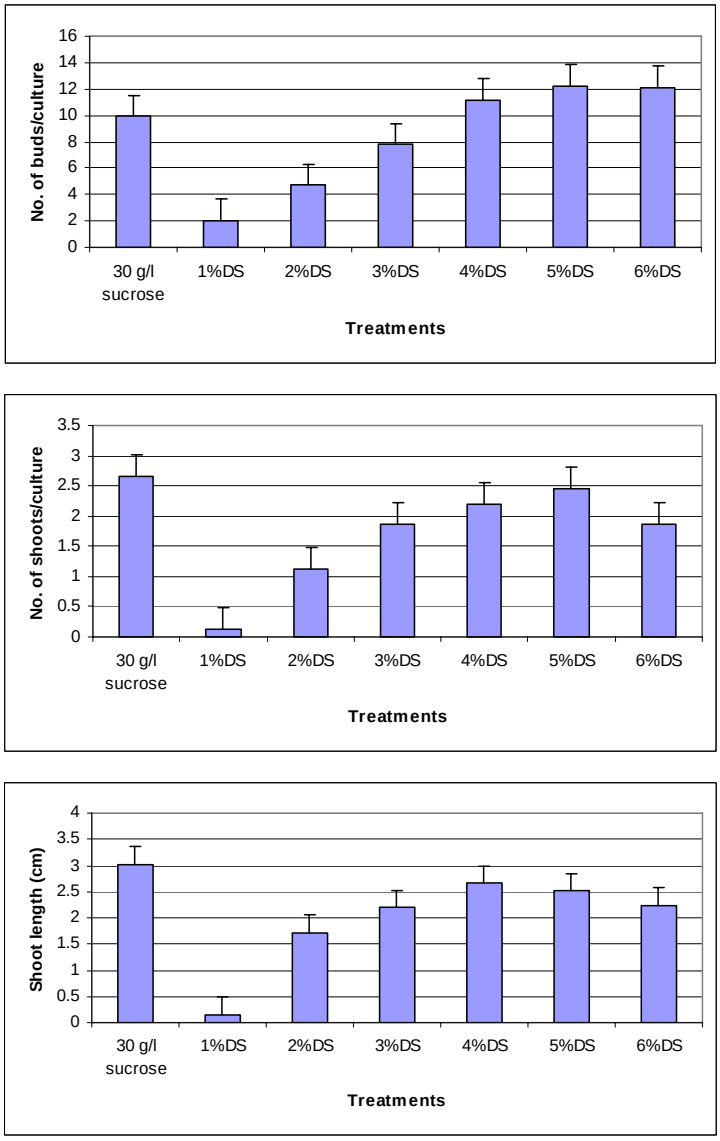


Fig (3): Number of buds and shoots/culture as well as length of longest shoot of date palm (*Phoenix dactylifera)* cv. Khanezi *in vitro* tissue culture as affected by different concentration of date syrup. Lines over bars represent LSD at 5%.

0 5% 10% 20%
(30 gl^{-1} sucrose)

Fig. (1): Effect of syrup (Dibs) of date palm on bud and shoot formation in flasks of cv. Khanezi date palm cultivar (note color media degree) in the first experiment.

0 5% 10% 20%
(30 gl^{-1} sucrose)

Fig. (2): Effect of syrup (Dibs) of date palm on bud and shoot formation in Khanezi date palm cultivar in the first experiment.

Table (1)

The constitution of date palm syrup

Components	Value
Moisture content (%)	**16**
Ash content (%)	**6.8**
Total solids on dry weight (%)	**84.0**
Total sugar (%)	**79.45**
Reduced sugar (%)	**4.87**
Invert sugar (%)	**74.83**
Total proteins (as N) (%)	**0.83**
Total lipids (fats) (%)	**1.98**
Pectin content (as calcium pectate) (%)	**1.46**
Vit. C. content (mg/100 g)	**0.185**
Minerals (mg/100 g)	
Sodium	**13**
Potassium	**202.8**
Iron	**7.8**
Magnesium	**143**
Calcium	**338**

Table (2)

Effect of date syrup in enhancing the growth of date palm (*Phoenix dactylifera*) cv. Khanezi *in vitro* tissue culture

Parameters	% Date Syrup*		
	Control (30 g l^{-1} sucrose)	5	10
Number of buds /culture	3.888a**	4.444a	0.888b
Number of shoots/culture	1.222a	1.000a	0.000b
Length of longest shoot/culture (cm)	2.211a	1.644a	0.000b
Percentage of rooting	0.444a	0.333ba	0.000b
Number of roots/culture	0.555a	0.666a	0.000b
Length of longest root/culture (cm)	0.411a	0.466a	0.000b
Fresh weight of culture (g)	2.122ba	2.652a	1.544b
Dry weight of culture (g)	0.228a	0.377a	0.318a

- *Since all plants were killed at 20% date syrup concentration, therefore this treatment was omitted from the table.
- ** Means in each column followed by the same letter(s) did not differ at < 0.50 according to Duncan's multiple-range test.

tissue culture of plants are needed. Such experiments would be beneficial to those who are interested in the production of plant tissue culture. If such experiments succeed, this will reduced the cost of the production of plants tissue culture.

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of tissue in the case of toxicity caused by high date syrup concentration. This dryness looks like as if the tissues have lost water.

In the present study the importance of sugars for bud formation was clearly shown as indicated by the reduction of bud formation in the medium supplemented with low date syrup concentrations 1 to 3% (Fig 3, 6 and 7). It seems that there was apparently insufficient sugar available in such concentrations of date syrup, which led to its reduction. In the future, adding 1 to 3% date syrup to the 30 g l^{-1} sucrose may be benefitial and enhance the shoot and bud formation of date palm.

The reason for the superior effect of the media with date syrup at 4 to 6 % was presumably due to the expend no energy to break down sucrose into monosaccharide. Al-Hooti *et al*. (2002) reported that glucose and fructose are the major sugars presented in date syrup and total sugar contents were reaching 88%. This is in accordance with findings that sucrose is degraded in to smaller units before uptake into cells in culture (Fowler, 1982). Alternatively date syrup contains in addition to sugar, macro and micro elements (Table 1) which may contribute to the enhancement effect of date syrup in micropropagation of date palm.

In this study, it is recorded that rooting percentage and the number of roots were enhanced (Fig 4) with increasing date syrup concentration. This raise the possibility that root formation required more energy than bud or shoot formation since shoot formation was enhanced under low date syrup concentration.

In conclusion**,** the results indicated that date syrup was taken up from the medium, as shown by the increase in total dry weight of culture (Fig 5). Date palm tissues are capable of utilizing date syrup as the sole carbon source for vegetative growth. Furthermore, date syrup at concentration of 4 to 6% can be used totally as a replacement of 30 g l^{-1} sucrose which was the normal sugar used in most of plant tissue culture**.** It is clear that the substitution of date syrup at 4 to 6% for 30 g l^{-1} sucrose clearly promoted the bud and shoot formation of date palm cultures and to a large degree, better than sucrose. It is well known that date syrup contains minerals and vitamins in addition to sugars (Table 1). Therefore, further experiments that look into the effect of date syrup and agar only on the

Warm White Weisse 3500 fluorescent tubes for both experiments. Each treatment was represented by 10 replicates and 2 buds per replicate in a completely randomized design. After 6 weeks from the onset of the experiment, number of buds and shoots per culture were determined. Also, shoot length was determined by measuring the longest shoot. After obtaining fresh weights of the cultures, it was placed in a forced air oven at 75°C for 72 hours to determine dry weights.

The data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the completely randomized design (Gomez and Gomez, 1984). The treatment means were compared using the least significant difference (LSD) at 5 % level of probability (Waller and Duncan, 1969).

Results and discussion:

The results shown in Table 2 indicates that date syrup at 5% concentration enhances number of buds, fresh and dry weight of culture as compared to control (sucrose 30 g l^{-1}). However, higher concentrations of 10 and 20% date syrup are detrimental for tissue growth (Table 2 and Fig 1 and). Tissues grown on medium containing 4 to 6 % date syrup had the highest number of buds and shoots, whereas tissues grown on medium containing 1 to 3% date syrup had the lowest. In addition, tissues grown on sucrose medium had vegetative growth rates similar to those grown on 4 to 6 % date syrup. Similar results were obtained in other studies related to adding of plant extracts juice of coconut, tomato, potato, onion, banana, orange, apple, pineapple and yeast to the culture medium (Chen *et al*., 2005; Lo *et al*., 2004; He *et al*., 2003; Hong *et al*., 2003; Amo-Marco and Picazo, 1994).

Most of the vegetative growth characteristics measured were enhanced with increasing date syrup in the medium up to 6%. However, with further increase of date syrup up to 20%, the growth of tissues was suppressed (Fig 2). This reduction of growth could be attributed to a supra-optimal effect caused by the more negative osmotic potential generated by the increase of date syrup. It is noted that the sever symptom of toxicity (Table 2 and Fig 1 and 2) caused by high syrup concentration is similar to that caused by high sugar concentration (Alkhateeb, 2007) except dryness

Although there are data on the effect of different plant extracts on *in vitro* plant culture, no data are currently available for the use of date syrup on date palm tissue culture or other plants. The objective of the present study was to investigate the the possibility of using date syrup locally known as 'Dibs' in tissue culture and to determine the optimum level of date syrup concentration that enhance the *in vitro* growth of date palm cv. Khanezi.

Materials and Methods:

These experiments were conducted in the Tissue Culture Lab. of the Date Palm Research Center, King Faisal University, Al-Hassa, Saudi Arabia. Date palm of cv. Khanezi approximately 3-years-old off-shoots and weighing 5-7 kg were separated from healthy mother palm. Offshoots were thoroughly cleaned and the outer leaves were carefully removed to expose the shoot tips and lateral bud. The exposed regions were excised and immediately placed in antioxidant solution containing 150 mgl^{-1} ascorbic acid and 100 mgl^{-1} citric acid. The shoot tip and lateral buds were sterilized in 20% sodium hypochlorite (domestic bleach) containing 2-3 drops of Tween-20 for 15 min, followed by rinsing 3 times with distilled water.

The shoot tips and lateral buds were sectioned into approximately 1 cm explants which were used for organogenesis culture as described by Alkhateeb and Ali-Dinar (2002). Two buds that resulted from direct organogenesis were transferred to 100 ml flasks filled with 50 ml of modified MS salts media (Murashige and Skoog, 1962) supplemented with 170 mg l^{-1} $NaH_2PO_4.2H_2O$; 125 mg l^{-1} inositol; 200 mg l^{-1} glutamine; 1 mg l^{-1} thiamine HCl; 1 mg l^{-1} pyridoxine HCl; 1 mg l^{-1} nicotinic acid; 1 mg^{-1} calcium pantothenate; 1 mg/l biotin; 7 g^{-1} purified agar; 0.2 mg l^{-1} kinitin; 0.1 mg l^{-1} 2ip; 0.1 mg l^{-1} NAA; 0.1 mg l^{-1} NOA and 0.1 mg l^{-1} IAA. The modified MS media was further supplemented with sucrose at a concentration of 30 g l^{-1} as control and different concentrations of date syrup (5, 10 and 20 %) in the first experiment.

Based on the results of the first experiment another one was carried out using different concentrations of date syrup that were 1, 2, 3, 4, 5 and 6%. Cultures were incubated at 25±2°C in 16 h of light daily supplied by 65/80

Introduction:

Date palm (*Phoenix dactylifera* L.) is the major fruit crop in the Kingdom of Saudi Arabia. Its ability to tolerate arid environmental conditions, made Saudi Arabia quite unique for its cultivation (Ibrahim and Khalif, 1998 and Alkhateeb and Ali-Dinar, 2002). The estimated annual production of the kingdom is 830000 tons from an area of 140000 hectares planted with date palms (FAO, 2004). It is well known that date palm is propagated sexually through seeds and vegetatively by offshoot (Alkhateeb *et al.*, 2006). Tissue culture is a technique mainly used for rapid propagation of several perennial fruit trees including date palm. Date palm is propagated *in vitro* by two methods; the first is by embryogenesis in which vegetative embryos are formed from embryogenic callus. The second is through organogenesis which produces date palm buds that eventually give plantlets without passing through the callus stage (Alkhateeb and Ali-Dinar, 2002).

It is well known in plant tissue cultures a continuous supply of carbohydrates is essential, because the photosynthetic activity of *in vitro* plant tissues is reduced due to low light intensity, high relative humidity and limited gas exchange (Kozai, 1991). Sucrose is the most widely used carbohydrate and carbon source in plant tissue culture as indicated by numerous studies (Hildebrandt and Riker, 1949; Vuke and Mott, 1987; Alkhateeb, 2001, 2007).

It has also been reported that in many plant species adding of plant extracts juice of coconut, tomato, potato, onion, banana, orange, apple, pineapple and yeast to the culture medium enhanced the growth of tissues (Chen *et al*., 2005; Lo *et al*., 2004; He *et al*., 2003; Hong *et al*., 2003; Amo-Marco and Picazo, 1994). Kinnersley and Henderson (1988) found that addition of corn syrup to the basic culture media improved embryogenesis of wild carrot. El-Assar *et al*. (2004) studied the effects of natural extracts of coconut water, date palm merstimatic tissues extract and casein hydrolysate on the growth of date palm cv. Sewi tissue culture grown *in vitro*. They found that date extract was superior to other natural extracts in producing growing tissues which were longer, larger in diameter and more highly coloured.

Enhancing the Growth of Date Palm (*Phoenix Dactylifera) in Vitro* Tissue by Adding Date Syrup to the Culture Medium

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Abstract:

The present experiments were conducted in the Tissue Culture Lab. of the Date Palm Research Center, King Faisal University, Al-Hassa, Saudi Arabia. Different concentrations of date palm syrup locally known as 'Dibs' (5, 10 and 20%) and sucrose (30 g l^{-1}) as control were used for *in vitro* multiplication of date palm cv Khanezi. Preliminary observations have shown that lower concentration of date syrup (5%) have successfully induced buds and shoots formation. Higher concentrations (10% and 20%) of date syrup, however, have resulted in browning and dryness of plant materials. This phenomenon was probably caused by osmotic stress due to the use of higher date syrup concentration . The results have also indicated that most of the parameters like number of shoots, fresh and dry weights of culture have increased when 5% date syrup was used compared to control (sucrose 30 g l^{-1}).

Based on the results of the first experiment another one was carried out using different concentrations of date syrup that were 1, 2, 3, 4, 5 and 6%. The results have shown that date syrup at concentration of 4 to 6% improved production of bud and shoots. In addition, tissues grown on medium containing 1 to 3% date syrup had the lowest number of buds and shoots. The results indicated that date syrup was taken up from the medium, as shown by the increase in total dry weight of culture. Date palm tissues are capable of utilizing date syrup as the sole carbon source for vegetative growth. Furthermore, date syrup at concentration of 4 to 6% can be used totally as a replacement of 30 g l^{-1} sucrose which was the normal sugar used in most of plant tissue culture. This is the first report on the use of date syrup on the multiplication of date palm cv Khanezi.

Key words:

date palm, *Phoenix dactylifera*, *in vitro*, sucrose, date syrup

تأثير تغير درجة الحرارة وسط النمو على تركيب جلايكوليبيدات الغشاء من الأحماض الدهنية في السيانوبكتيريا Aphanizomenon sp. من نوع

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الملخص :

في هذة الدراسة تم تتبع تركيب الاحماض الدهنية و درجة عدم التشبع عند تحول درجة حرارة النمو من ٢٨°م الى ١٥°م و من ١٥°م الى ٢٨°م في السيانوبكتيريا من نوع *Aphanizomenon sp.* . إن تحول درجة حرارة من ٢٨°م الى ١٥°م قد أدى إلى تغير كلي في تركيب الاحماض الدهنية في الليبيدات الكلية و الجليسروليبيدات مع إستجابة سريعة و بطيئة في محتوى الاحماض الدهنية أثناء فترة الاقلمة. إن الاستجابة السريعة كانت محصورة على ١٢ ساعة التي تلت تحول الحرارة قد شوهدت في جزء PG حيث وجد أن C18:1 قد زاد بحوالي إثنان و نصف مرة بينما مستوى C16:0 و C18:3 قد إنخفض و الأستجابة السريعة نسبيا قد وجدت في إنخفاض C16:0 مع زيادة متلازمة في C18:3 في MGDG و DGDG . و مقارنة تركيب الاحماض الدهنية عند ٢٨°م (الزمن صفر) مع تلك عند نهاية فترة النمو على ١٥°م (٤٨ ساعة) أوضحت أن معظم المعنوية هي زيادة نسبة C18:3/C18:2 في الليبيدات الكلية و MGDG و SL . و أوضحت النتائج أيضا زيادة في درجة عدم التشبع في أجزاء الليبيدات الكلية و MGDG و SL بينما لا يوجد تغيير في DGDG و إنخفاض في جزء PG .

عند تحولت درجة حرارة النمو من ١٥°م الى ٢٨°م فإن نمط التغيير في تركيب الاحماض الدهنية و درجة عدم التشبع مخالفة للتي شوهدت عند تحول درجة حرارة النمو من ٢٨°م الى ١٥°م . فقد حدث إنخفاض C16:3 و زيادة محسوسة في C16:1 في الليبيدات الكلية و MGDG و PG و SL . في الليبيدات الكلية لوحظ إنخفاض صغير في C18:3 الذي صاحبه زيادة مضاعفة في C18:1 . مع ذلك فإن كمية C16:0 و C18:0 ظلت ثابتة أثناء الأقلمة لدرجة الحرارة .

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Table (1)

Effects of growth temperature shift from 28°C (0hr.) to 15°C (48hr.) on the degree of S saturation/unsaturation as determined by the average number of double bond per lipid molecule.

Fatty acid composition (mole %)										
		C16:0	C16:1	C16:2	C16:3	C18:0	C18:1	C18:2	C18:3	Average No. of double bond per
Total lipid	28° (0hr.)	37.60	13.55	2.60	4.10	3.15	11.45	6.50	22.00	2.43
	15° (48hr.)	31.20	15.25	2.05	7.15	1.65	7.60	4.10	31.15	3.001
MGDG	28o (0hr.)	30.25	20.80	3.70	7.20	2.09	4.85	5.20	25.20	2.813
	15° (48hr.)	26.45	15.95	2.50	10.30	1.05	2.75	2.35	38.80	3.514
DGDG	28° (0hr.)	33.35	12.10	1.40	6.15	3.55	10.45	5.50	27.65	2.755
	15° (48hr.)	38.75	11.20	2.10	4.10	2.50	5.95	5.59	30.15	2.72
PG	28° (0hr.)	35.45	11.15	0.00	0.00	4.00	10.05	6.00	27.55	2.317
	15° (48hr.)	32.05	13.75	0.00	0.00	5.25	23.40	1.90	19.90	2.013
SL	28° (0hr.)	53.60	6.15	0.00	0.00	6.40	15.95	7.00	9.95	1.319
	15° (48hr.)	49.00	9.40	0.00	4.05	4.45	9.35	5.10	18.45	1.929

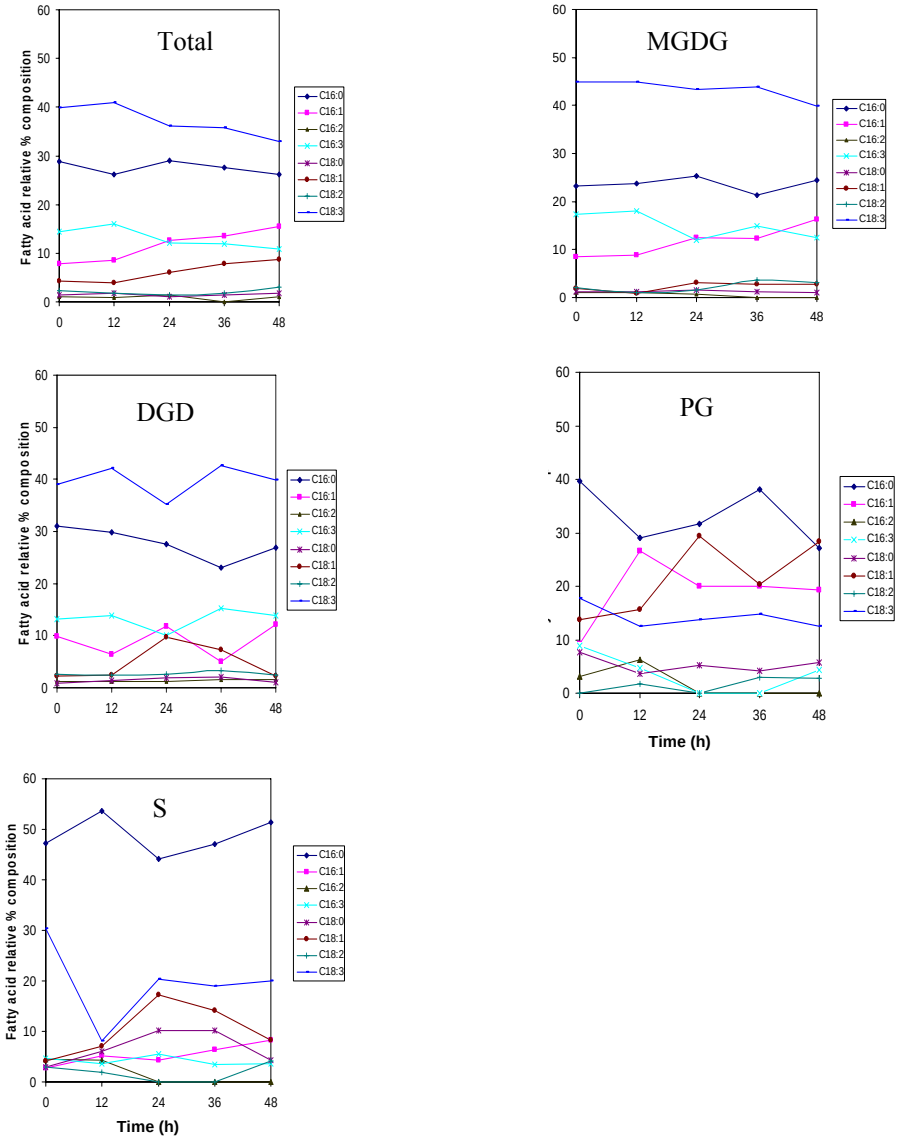

Fig. (2) : Relative percentage composition of major fatty acids in Total Lipid, MGDG, DGDG, PG and SL fractions from *Aphanizomenon sp.* Culture following a temperature shift from 15°C to 28°C.

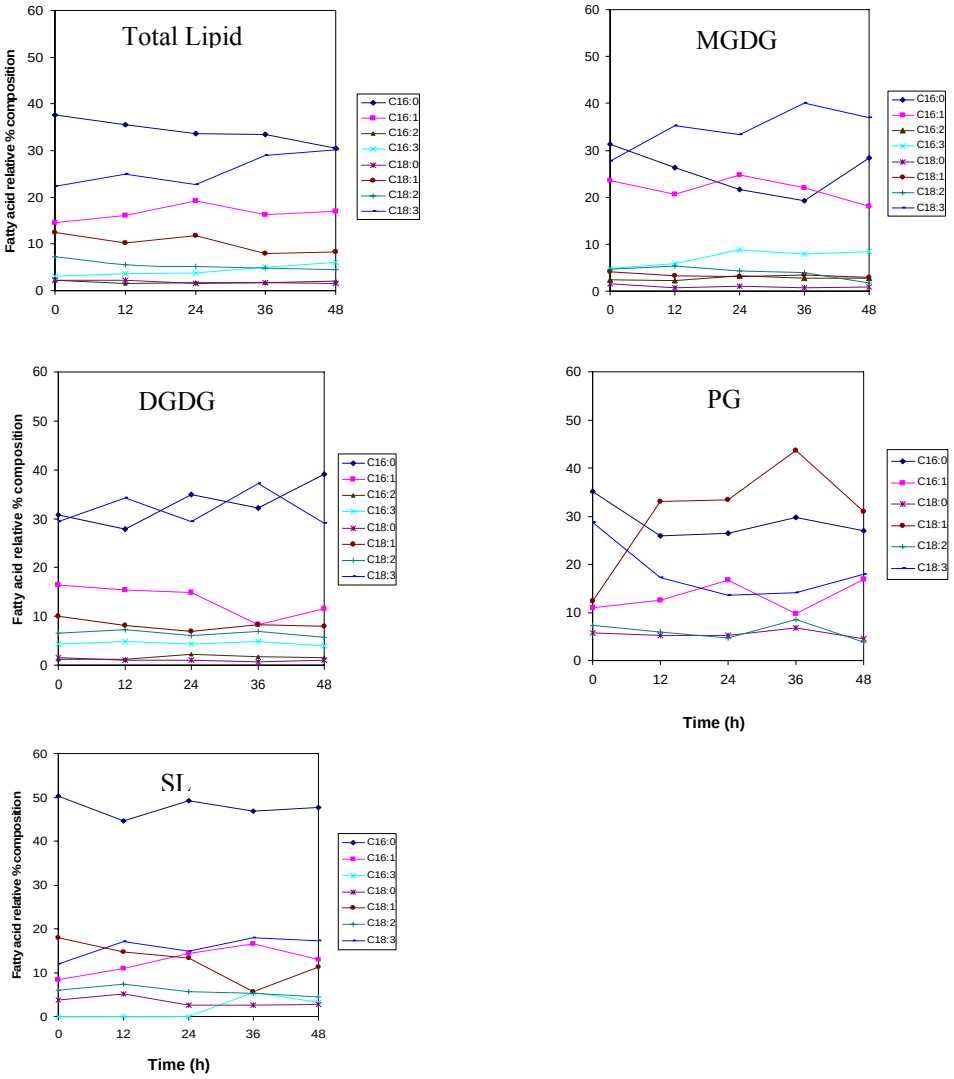

Fig. (1) : Relative percentage composition of major fatty acids in Total Lipid, MGDG, DGDG, PG and SL fractions from *Aphanizomenon sp.* Culture following a temperature shift from 28°C to 15°C.

C18:3 was observed in all of the lipid classes which was similar to that found in *Aphanizomenon sp*. The results observed during the temperature shift from 15^{o}C to 28^{o}C concluded that there is a decrease in the level of unsaturation similar to that found in other cyanobacteria species. But that the increase in saturation level may be brought about by different mechanisms from those observed previously.

that saturation during initial adaptation to higher temperature could induce partially by conversion of C16:3 to C16:1.

After 48h the temperature shift from 15°C to 28°C (Table2) caused an increase in the level of saturation in the fatty acyl chains of the membrane glycerolipid, as the average number of double bond per fatty acid molecule in the total lipid decreased by 14.563% (decreased from 3.296 to 2.816). Among C-16 acids, there is no evidence for an increase in the proportion of C16:0, but a decrease in C16:3 and a marked increase in C16:1 were observed in total lipid, MGDG, PG and SL. Also, changes in C-18 fatty acids were observed. In the total lipid small decrease (17.08%) in C18:3 was found, accompanied by an approximate doubling in the proportion of C18:1 within 48h of the upward temperature shift, and the level of C16:3 also decreased by 25% whilst C16:1 increased by 96.2%. The amount of saturated C-16 and C-18 fatty acids however, remained constant during temperature adaptation. These finding suggested that the observed changes in fatty acid composition are most probably due to the accelerated syntheses of C16:1 and C18:1 and substitution of the preexisting polyunsaturated fatty acid by newly synthesized fatty acids.

Similar changes were obtained in MGDG following the temperature shift from 15°C to 28°C. The saturated fatty acids remain unchanged whilst C18:3 and C16:3 decreased by 11.1% and 27.7% respectively, and C16:1 increased by 91.76% and C18:1 by 42.1%. The similarity in the fatty acid patterns and changes observed during temperature adaptation in the two galactolipid fraction was consisted with the conversion of MGDG to DGDG by galactosylation during the acclimation to higher temperature, as has been suggested previously (Sato and Murata,1980). The acidic lipid SL contained a substantial proportion of C16:0 which remained unchanged following the upward temperature shift. Moreover, the trend of the fatty acids present in PG was similar to that in SL. The responses seen in the glycoglycerolipid C-16 fatty acids of *Aphanizomenon sp*. are different from those reported by Sato and Murata (1980), who found that in the first 5h after temperature shift from 22°C to 38°C in *A. variabilis*, the relative content of C16:0 increased whilst C16:1 decreased specifically in MGDG and DGDG. Also in *A.variabis*, a decrease in the proportion of

of C18:1 (increased almost 205 fold) in PG during the first 12h and drop in C18:3 during the first 24h. This observation appears to indicate that de novo synthesis of C18:1 may be an important adaptive mechanism in retailoring this lipid class.

Table 1 present a comparison of fatty acid composition at 28^{o}C (0h) with those at the end of the 15^{o}C growth period (48h). The most significant changes were the increased linolenic (C18:3) to linoleic (C18:2) acid ratio in total lipid (from 3.054 to 6.68), MGDG (from 5.87 to 21.76) and SL (from 1.98 to 3.91). This increased of unsaturation of fatty acids at low temperature can be attributed to a direct (Mazliak, 1981) or indirect (Kiseleva *et al*. 1999) effect of temperature on fatty acid saturation activities.

Table 1 also shows the effects of temperature change from 28^{o}C to 15^{o}C on the degree of unsaturation of total lipid and glycerolipid classes. We observed an increase in the average number of double bond per lipid molecule in the total lipid and MGDG and SL fractions, while there is little change in DGDG and decrease in PG fractions. In general, in microorganisms, the fatty acid composition of membrane glycerolipid is dependent on growth temperature (Lynch and Thompson, 1982). Increase in the level of degree of unsaturation in fatty acids is considered to provide a mechanism for the thermoadaptive regulation of membrane lipid fluidity (Mortensen *et al*., 1988). Previous reports have suggested that decreasing temperature cause a general increase in the degree of unsaturation of fatty acids in marine phytoplankton (Pohl and Zurheide, 1979; Harwood and Russel, 1984). We suggest that in *Aphanizomenon sp*. that C18:1 serve partially as modulator of low temperature adaptation.

Effect of Increasing in Growth Temperature From 15^{o}C to 28^{o}C on Fatty Acid Composition

Fig. 2 shows the pattern of changes in fatty acid composition in the total lipid classes upon temperature shift from 15^{o}C to 28^{o}C. The most significant change was observed in the shooting 0f C16:1 in PG and drop in C16:0 during the first 12h whilst over the same period C16:3 was decreased to 53.4% of its original value. These observations may suggest

of interest in the chromatogram. Absolute quantification of FAME was obtained by injection of known amounts of an appropriate authentic FAME standard and determination of peak area. The amount of unknown FAME was then calculated by proportionation.

The degrees of saturation were calculated from the relative amounts of fatty acids in each lipid class as the values of the average number of double bonds per lipid molecule. For example value of zero with only saturated fatty acid and a value of 2 for C16:1, and C18:1, and a value of 4 for C16:2, and C18:2, and a value of 6 for C16:3, and C18:3(Havaux *et al*.,1984).

Result and Discussion :

Effect of Reduction in Growth Temperature From 28^{o}C to15^{o}C on Fatty Acid Composition

Figure 1 shows the time dependant changes in fatty acid composition in total lipid and glycerolipid classes when the temperature shifts from 28^{o}C to15^{o}C. At 28^{o}C (0h) MGDG and DGDG had almost a similar fatty acid composition and contained polyunsaturated C-16 and C-18 fatty acids, whilst in the acidic glycerolipid PG and SL, there was no polyunsaturated C-16 fatty acids, and the level of C18:3 in SL was generally lower than in galactolipid fractions. Temperature shift from 28^{o}C to15^{o}C led to changes in the overall fatty acid composition in total lipid and glycerolipid classes. The changes observed in the fatty acid composition of the total lipid after lowering the temperature were broadly similar to those observed in the MGDG and DGDG fractions, where the reduction of growth temperature led to decrease in C16:0 and C18:1 while C16:3 and C18:3 increased. In contrast PG fraction the level of C18:1 increased whilst the level of C18:3 decreased. In SL fraction C16:0 was the dominant fatty acid and its level decreased slightly, whilst C16:1 increased, and a fall in C18:1 level was accompanied by an increase of C18:3.

The results in Fig.1 demonstrate the presence of relatively rapid and slow responses in the mechanisms by which the fatty acid content is modulated temperature acclimation. The most significant change upon shifting the temperature from 28^{o}C to 15^{o}C was observed in the shooting

H_2O (4: 1, v/v), and visualized under UV light, or visualized by iodine as described by Christie (2003).

Fatty acid methyl esters (FAME) of total lipid fractions and isolated glycerol lipid fractions were prepared by transesterification. Samples (100 – 200 µl) of total lipid extracts or isolated glycerolipid fractions were evaporated to dryness under oxygen free nitrogen (OFN), and then heated at 55^{o}C for 15h with 2ml 2% H_2SO_4/ absolute methanol (Christie,2003) and extracted by petroleum ether.

The methyl ester samples prepared by transesterification of the total lipids fractions and glycerolipid classes were purified by preparative TLC on Whatman 60A silica gel-G coated plates. An authentic solution of fatty acid methyl ester standard (5mg/ml) was used for identification of the sample methyl esters. A solvent system of diethylether: petroleum ether (1: 9, v/v) was used to develop the chromatogram. Developed plates were sprayed with 0.05% (w/v) primulin in acetone/water (4: 1, v/v), visualized under UV light (340nm), and detected fatty acid methyl esters were removed from the plates by extraction in petroleum ether. The recovery of FAME from transesterification and preparative TLC was estimated by adding a known amount of internal standard heptadecanoic acid to representative total lipid extract before transesterification, then processing as described above. An apparent recovery of 73% was routinely obtained. Preparation of fatty acid methyl esters for gas chromatographic analysis of marine lipids: insight studies.

FAME recovered from TLC plates were identified and quantified by GLC (Perkin Elmer-8700 gas liquid chromatography, Boston, MA,USA) equipped with a flam ionization detector using a highly "polar" capillary column (BPX70, 0.25µm film, 25m x 0.22mm id). Oven temperature was programmed from 130^{o}C to 210^{o}C at a rate of 20^{o}C min^{-1}. Injections were made in the split injection mode (10: 1) at an injection temperature of 160^{o}C using helium as carrier gas at a flow rate of 10ml min^{-1}. Individual components were identified by comparison their retention times with authentic FAME standards and by comparison with a GLC-MS total ion chromatogram determined under similar conditions. The relative % composition of fatty acid was calculated from area of the individual peaks

for 15 minutes. When the flask attained room temperature, it was inoculated. Normally, 10% by volume of inoculum was used from a culture which had previously been grown for 10 – 12 days. Inoculations were carried out by sterile transfer in a console safety cabinet. Inoculated flasks were placed in an illuminated refrigerated orbital incubator on a 12 h light (90 μE $m^{-2}s^{-1}$) /12 h dark cycle , the speed of shaking was 12 rpm and normally culture were maintained at a growth temperature of 28°C.

Aphanizomenon sp. culture was grown in 1500 ml of modified ASM-1 medium (Rapala *et al*., 1997) contained in 2L conical flask for 10 days at 28°C as described above. The incubator temperature was then reduced to 15°C. Under these conditions the temperature of culture in the incubator were found to have fallen to 15°C within 1.5 h of the incubator temperature being lowered. Culture was maintained at 15°C for 48h, during which time samples of culture were collected at 0h (immediately before lowering the temperature), then at 12h, 24h, 36h and 48h for the analysis of fatty acid composition. In another experiment, the organism was grown at 15°C for 10 days, then the temperature of the incubator was raised to 28°C and growth continued for 48h. Samples of culture were collected at the time the temperature was raised (0h) to28°C and after further periods of 12h, 24h, 36h and 48h for fatty acid composition analysis.

Analysis:

Total lipid was extracted from *Aphanizomenon sp*. cells which had been harvested by centrifugation, normally at 3,000xg essentially according to the method of Bligh and Dyer (1959) as modified by Murata and Sato (1981).

For separation of glycerolipid classes were done by the method of Harwood *et al*. (1988), in which the total lipid extracts was applied as a short band to silica gel-G TLC plates together with authentic samples of MGDG, DGDG and PG. The chromatogram was developed in a solvent of chloroform: methanol: acetic acid: water (170: 30: 20: 7, v/v/v/v). Developed plates were sprayed with 0.05% (w/v) primulin in acetone/

onto the water surface(Evans *et al*., 1996, 2000; HELCOM, 2002; Laanemets *et al*., 2004).

In summer when the water column is thermally stratified, wind-driven coastal upwelling is an important mesoscale phenomenon that dramatically changes the euphotic layer temperature and nutrient conditions in the Baltic Sea. The surface-layer water temperature may drop more than ten to fifteen degrees within hours, while nutrient concentrations increase markedly (Vahtera *et al.*, 2005). The solar heating and wind mixing form a sharp seasonal thermocline at a depth of about 10–15 m starting from May. The seasonal thermocline is the strongest in July/August, when the temperature difference between the warm upper mixed layer and the cold intermediate layer varies within 12–20°C (Laanemets *et al.*, 2004). In the Baltic, *Aphanizomenon sp*. and other cyanobacteria are exposed during the summer to a wide range of temperature from a maximum of 30^{o}C whilst floating at the surface under calm condition to a minimum of 10^{o}C when they sink to the thermocline following a wind induced mixing event (Helsinki Commission, 2006). The aim of this study is to determine change in fatty acid composition in the total lipid fraction and major glycerolipid classes of *Aphanizomenon sp*. following (i) a downward temperature transition from 28^{o}C to 15^{o}C, and (ii) an upward temperature shift from 15^{o}C to 28^{o}C.

Materials and Methods :

Growth and maintenance of laboratory cultures

A culture from a strain of *Aphanizomenon* originally isolated from the Gulf of Finland by Dr Kaarina Sivonen, University of Helsinki, Finland, was obtained from the Biochemistry Research Group, University of Wales Swansea , henceforth referred to as *Aphanizomenon sp.*, and was grown photoautotrophically in modified ASM-1 liquid medium lacking a fixed nitrogen source (Gallon et al., 1978). The medium ingredients were mixed thoroughly and the pH value of the medium was adjusted to 7.6 by adding of small volume of 0.1 M HCI. Volumes of the medium were transferred into conical flasks , typically to 1\2 – 3\4 of the flask volume, which were then loosely plugged with non-absorbent cotton wool and sterilized in an autoclave at 121^{o}C at pressure of 1.5 kg-force/cm^{2}

found by Kenyon *et al*. (1972) and confirmed by Murata *et al*. (1992). The fifth group described by Cohen *et al*. (1995) was positioned according to the Kenyon-Murata classification system between groups 1 and 2. The strains of group 1 are devoid of polyunsaturated fatty acid (PUFA) and contain only saturated and monounsaturated fatty acids. The strains of the group defined by Cohen *et al*. (1995) contain 18:2Δ9,12 (18:2ω6, linoleic acid) as the only C_{18} PUFA. Group 2 consists of cyanobacterial strains containing 18:3 Δ9,12,15 (18:3ω3, *a*-linolenic acid) as the only C_{18} PUFA. The strains of group 3 have 18:3Δ6,9,12 (18:3ω6, *γ*-linolenic acid) as the major C_{18} PUFA, but no or only traces of 18:3Δ9,12,15. Strains of group 4 contain either 18:3Δ9,12,15 or 18:3Δ6,9,12 or both, but also produce 18:4Δ6,9,12,15 (18:4ω3, octadecatetraenoic acid). The double bonds in the hydrocarbon chains of PUFA are introduced by fatty acid desaturases, which play an important role in the acclimation of the various organisms to changes in environmental temperatures (Murata and Wada, 1995; Nishida and Murata, 1996).

In the Baltic Sea, high abundances of planktonic cyanobacteria are common during the summer months (Stal *et al.*, 2003). The dominating genera are two photoautotrophic filamentous diazotrophs (*Nodularia* and *Aphanizomenon*) that use heterocysts in the process of assimilating nitrogen from the environment through N_2 fixation (Gallon *et al.*, 2002; Janson & Granéli, 2002). Early studies showed that they occur at the most nutrient-deplete time of the year, during an approximately two month long time-window from the end of June to the end of August when surface-layer temperatures are high (Wasmund, 1997). These cyanobacteria form colonies or larger aggregates that float to the water surface where they accumulate as dense scums, because they contain gas vesicles, (Walsby *et al*., 1995, 1997). Despite the fact that these cyanobacterial blooms occur locally and for relatively short periods (Evans *et al.*, 2000), *Aphanizomenon* sp. is abundant in the water mass during the whole year, thus showing the ability to grow in low or high temperatures (Niemistö *et al.*, 1989). *Aphanizomenon* exhibit maximum growth at temperatures of 12–25°C at photon irradiance levels of 25–45 μmol m^{-2} s^{-1}, and are more homogeneously distributed through the mixed layers and forms scums

Abbreviation: C16:0 palmitic acid; C18:1 oleic acid; C18:2 linoleic acid; C18:3 linolenic acid; MGDG monogalactosyl diglyceride; DGDG digalactosyl diglyceride; PG phosphatidyl glycerol; SL sulfoquinovosyl diglyceride; TLC thin layer chromatography; FAME fatty acid methyl esters; GLC gas liquid chromatography.

Introduction:

Biological membranes are not static structure, but can have a degree of fluidity in their interior. Membrane fluidity or bilayer order has a major influence over a wide variety of membrane functions and processing (Morgan-Kiss *et al.*, 2006). The extent of fluidity of the membranes depends on the degree of unsaturation of the fatty acid estrified to the glycerol backbone of the glycerolipids of the membrane (Veatch and Keller 2005). Resent research has focused on the cellular membrane as the primary site of low temperature injury by reducing the membrane fluidity (Lee *et al.*, 2006). An organism – in particular poikilotherms – may maintain the level of molecular motion or "fluidity" of its membrane lipid by regulating the number of double bonds in the fatty acids of these lipids (Russell, 1984; Jones, 2003). When the fluidity of membrane lipids is reduced by decrease in temperature, cyanobacteria and plants respond by introducing double bond into the fatty acids of lipids, so that membrane returns to more fluid state (Vigh *et al*., 1993).

Fatty acid composition of many organisms is affected when their normal environmental growth temperature either decreases or increases (Wada and Murata, 1990; Sushchik *et al.*, 2001). These modifications in glycerolipid fatty acid composition represent adaptation of membrane fluidity that enable the organism to carry out normal physiological function at either decreased or increased environmental temperature. In fact, cellular fatty acid composition has been used as a tool for classifying bacteria at the family, genus and species levels (Vandamme *et al*., 1996). Unicellular and filamentous cyanobacteria have been grouped in five clusters depending on the number and position of double bonds counted from the carboxyl terminus (Δ) or from the methyl terminus (ω) of 16 carbon (C_{16}) and 18 carbon (C_{18}) fatty acids (Kenyon, 1972; Kenyon *et al*., 1972; Murata *et al*., 1992; Cohen *et al*., 1995). Four groups were first

Effect of Environmental Temperature Transition on Fatty Acid Composition of Membrane Glycerolipids in Marine Cyanobacterium *Aphanizomenon sp*.

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Abstract:

In this study the fatty acid composition and the degree of unsaturation were followed upon temperature shift from 28°C to15°C and from 15°Cto 28°C. Temperature shift from 28°C to 15°C led to changes in the overall fatty acid composition in total lipid and glycerolipid classes, with rapid and slow responses in the fatty acid content during the adaptation period. The rapid response-limited to the 12h following temperature transition-observed in PG fraction in which C18:1 increased, and almost 2.5 fold, while C16:0 and C18:3 level decreased, and a relatively rapid response was presented by decrease in C16:0 and concomitant increase in C18:3 in MGDG and DGDG. A comparison of fatty acid composition at 28°C (0h) with those at the end of the 15°C growth period (48) shows that the most significant changes were the increase of C18:3/C18:2 ratio in the total lipid, MGDG and SL. The results also show an increase in the degree of concentration in the total lipid, MGDG and SL fractions, while there is no change in DGDG and decrease in PG fraction.

When the growth temperature shift from 15°C to 28°C, the pattern of the change in fatty acid composition and the degree of unsaturation were relatively opposite to that observed upon temperature shift from 28°C to 15°C. A decrease in C16:3 and a marked increase in C16:1 was observed in total lipid, MGDG, PG and SL. In the total lipid small decrease in C18:3 was found accompanied by a doubling increase in C18:1. The amount of C16:0 and C18:0 however remained constant during temperature adaptation.

Key word : Baltic Sea; *Aphanizomenon sp*.; temperature; fatty acids; glycerolipids; Plasma membrane;

نظام قياسي ضبابي لفهرسة قواعد بيانات أنظمة المراقبة عن بعد

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الملخص:

نقترح في هذا البحث بناء نظام استرجاع مرئي من قواعد بيانات تحتوي على فيديو مضغوطة MPEG خاصة بنظام مراقبة خارجية. الهدف هو تحديد الأشياء المتحركة. بعد ذلك يتم تصنيف هذه المناطق المتحركة إلى فئات محددة وفق الخصائص الأساسية للصور والمؤشرات القوية الموجودة فيها.

الفكرة الرئيسية التي تم تطبيقها هي الجمع بين الخاصيات القياسية ومفهوم التماثل الزمني. تكمن خصوصية طريقتنا في استخدام مفاهيم الهندسة الضبابية التي تضمن أحسن أداء في عملية تصنيف الأشياء المتحركة إلى ثلاث فئات: فئة المترجلون, فئة السيارات و فئة الأشياء غير المعروفة. و قد تم اقتراح التماثل الزمني كطريقة فعالة لمعالجة التغييرات التي تحدث على مظهر الأشياء المتحركة من جهة ومن جهة أخرى لإيجاد الحلول الفعالة للمشاكل التي قد تحدث نتيجة تداخل و تطابق هذه الأشياء المتحركة. تستعمل الأشياء المتحركة التي وقع تصنيفها كأدوات قياس على مستوى خوارزميات التدريب القياسي وأيضا كمفاتيح في بناء تقنية الفهرسة الشجرية.

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Fig. (5) : Searching the sequences that contain mobile pedestrian (a), mobile vehicle and pedestrian (b). Vehicle (c and d).

Conclusion :

The work presented here is concerned with motion region detection, classification and indexing moving regions from MPEG surveillance video sequences. The first stage is to decompose the video sequences into shots saving unnecessary decompression. Then, a set of representative frames is selected. The representative frames of a shot are used to the image pre-processing stage in order to generate a collection of moving regions of interest. A robust fuzzy system is proposed to classify moving regions into predefined categories; humans and vehicles, according to image-based properties. Classification is based on simple rules whish are largely independent of appearance or 3D models. Consequently, the metrical classification whish is explored in this paper, is based purely on object's shape, and not on its image content. An additional hypothesis on temporal consistency is used to make the classification system robust to changes of objects appearance and occlusion of motion regions. However, some problems remain to solve: it is necessary to study the problem that when multiple humans close together and when a target is very small.

wind are completely rejected. Furthermore, the accuracy of the classification is largely independent of target size, appearance shape, speed, lighting conditions or viewpoint. It is also computationally inexpensive. However, when multiple humans close together for a long time, they can be misclassified as a vehicle according to the simple metric. Another limitation of the system is that if the target is very small, less than 4x4 pixels, it tends to be rejected as no identified object. The main problem with vehicles recognition is that when, vehicle is partially occluded for long times, it could be rejected. Also, pedestrians tend to move in close groups that can be misclassified as vehicles according to the simple metric. Fig. 4 and 5 show some results of the system.

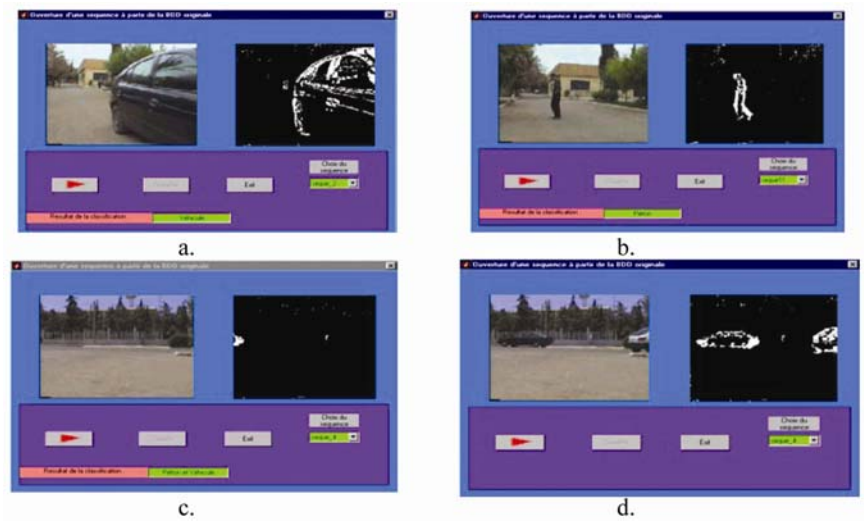

Fig. (4) : Sequences from the ICEPS Laboratory Database automatically segmented and classified as vehicle regions (a), pedestrian regions (b), pedestrian and vehicles regions (c and d)

Table (1)
Results of the learning algorithm

Class	Vehicle	Pedestrian
Dispersion	[17 45]	[23.2 125]
Dispersion Concentration	[20 30]	[30 60]
Ratio	[0.1 1.1]	[0.59 4.47]
Ratio Concentration	[0.2 0.7]	[1.9 3.2]

Indexing and Retrieval

Indexing digital video, based on its content, can be carried out at several levels of abstraction, beginning with indices like the video program name to much lower level aspects of video like the specified motion objects and their locations of the video [Idr 97] [Ray 96].

The interactive retrieval system proposed in this paper includes a query interface sub-module and a query by content retrieval sub-module as shown in figure 1. To facilitate storage and retrieval in visual information systems, flexible data structures should be used. Structures such as R-tree family, R^*-tree, quad-tree, and grid file are commonly used. Each structure has its advantages and disadvantages; some have limited domains and some can be used concurrently with others. To achieve a fast retrieval speed and make the retrieval system truly robust, a quad-tree indexing technique is applied [Khe 04, 05]. The goal of the system is to be able to retrieve a set of sequences, which have motion objects similar to that specified by the query. Reference frames are stored in the database to represent each shot. Image indexing is then applied on the referenced frames. Each frame is indexed according to the descriptors of its moving objects that are defined in the section 4. The metrical features are used as a primary indexing and querying elements

Results :

The system has been applied to large amounts of different video environments where human and vehicular activities are present. Fig. 4 shows some examples of target classification. For single targets, the system provides a robust classification. Note that trees blowing in the

used. The main idea is to record all potential motion regions PR_n from the first frame of the shot. Each one of these potential regions must be observed along some frames of the shot to determine if they persist or not, and so decide to continue classifying them. To do this, for each new frame, each previous motion region PR_{n-1} is matched to the spatially closest current motion region R_n according to a mutual proximity rule. After this process, each previous potential motion region PR_{n-1} whish have not been matched to current region are removed from the list of accepted motion regions. And any current motion region R_n whish has not been matched is considered new potential region. The metric operators, dispersion and ratio of each frame, are used to update the classification hypothesis [Khe 04]. The most advantage of this method is that if an occluded object is misclassified it will be correctly classified with the passage of time. Another advantage is that the instable motions appearing at the background, such as leaves blowing in the wind, will be misclassified as no-identified regions.

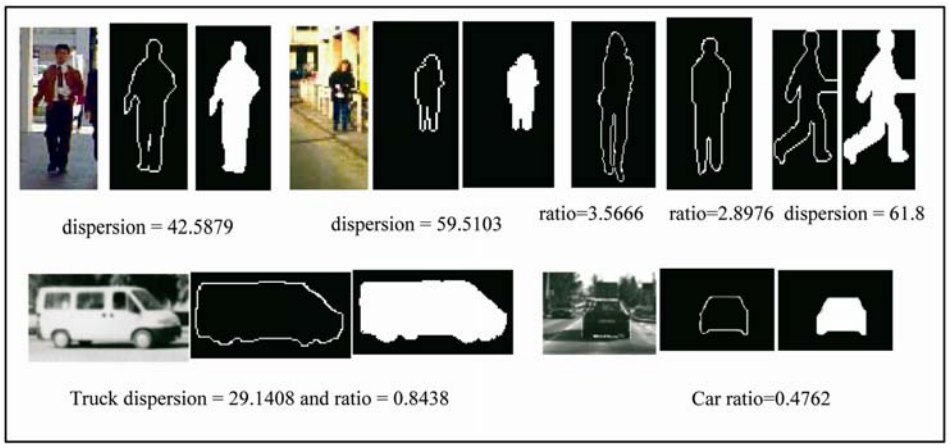

Fig. (3) : Human and vehicles dispersion/ratio values calculated for some image of the learning database.

Perimeter of a fuzzy set:

$$p(\mu)=\sum_{m=1}^{M}\sum_{n=1}^{N-1}\left|\mu_{mn}-\mu_{m,n+1}\right|+\sum_{n=1}^{N}\sum_{m=1}^{M-1}\left|\mu_{mn}-\mu_{m+1,n}\right| \quad (5)$$

Where M and N are the dimensions of the image.

Based on the perimeter and the area, the dispersion and the ratio of a fuzzy set can be determined as follows:

$$Dispersion = \frac{(Perimetre)^2}{Area} \quad (6)$$

$$Ratio = \frac{Length}{width} \quad (7)$$

The classified motion regions are used as templates for metrical training algorithms. The strategy adopted in this paper is to find the parameters of a fuzzy system by means of learning method obtained from neural networks. A common way to apply a learning algorithm to a fuzzy system is to represent it in special neural-network architecture and train the system using a learning algorithm, such as back propagation.

The fuzzy system is based on two entrances: the dispersion and the ratio of the motion regions, and one exit. For every entrance, we have two fuzzy sets: one for the category of humans and other for the category of vehicles. We have three fuzzy sets for the entrance: one for the human, one for the vehicle and one for no identified objects.

The system leads good performances (98%) over databases of 270 examples where 116 are pedestrians, 124 are vehicles and the rest represent states that are no identified (table 1).

The accuracy of the classification is largely independent of target size, appearance shape or speed. However, the main difficulty with metrical classification is that: when multiple humans close together, they can be misclassified as a vehicle according to the simple metric, if the target is very small, it tends to be rejected as no identified object, and a partly occluded vehicle may look like a human, or some background clutter may appear as a vehicle. To overcome this problem, an additional hypothesis is

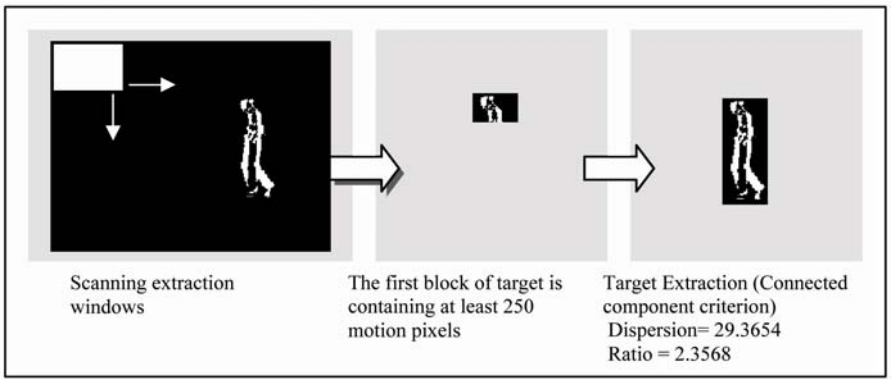

Fig. (2) : Grouping moving objects into motion regions using a connected component criterion.

Fuzzy Motion Region Classification System :

The task of the system is to distinguish the cars from pedestrians from other moving and stationary objects like animals, trees, roads and buildings in the image sequences and identify them as vehicles, human or non-identified object. The principal idea is to exploit useful properties of fuzzy metrical classification in order to provide a robust method to classify motion regions. Indeed, the regions are not always crisply defined, it is sometimes more appropriate to regard them as fuzzy subsets of the image [Bar 92] [Bez 92] [Dzu 01] [Rud 94] [Tiz 97]. The motivation of the use of the geometry features is that is computationally inexpensive and invariant to lighting conditions. On the other hand, it is obvious that the human, with its small and more complex shape, will have larger dispersion than a vehicle (figure 3).

If we define an appropriate membership function μ for the object [Khe 04], the area *a* and the perimeter *p* of the object can be calculated as follows:

Area of fuzzy sets:

$$a(\mu) = \sum \mu \qquad (4)$$

Firstly each I frame of a shot is smoothed with the second derivative in time of the temporal Gaussian function. If *fn* is the intensity of the n^{th} I frame of the shot, then the absolute difference function Δ_n is:

$$\Delta_n = | f_n - f_{n-1} | \quad (2)$$

The result of the difference is binarized in order to separate changed pixels from others. To do this, a threshold function is used and a motion image M_n can be extracted.

$$M_n(u, v) = \begin{cases} f_n(u,v) & \text{if } \Delta_n(u,v) \geq T \\ 0 & \text{if } \Delta_n(u,v) < T \end{cases} \quad (3)$$

Where *T* is an appropriate threshold chosen after a several tests according to the exterior environment with different acquisition conditions [Khe 03].

To separate the regions of interest from the rest of image, binary statistical morphological operators (erosion and dilatation) are used. This allows decreasing the number of connected components. Then, the moving sections must be grouped into motion regions $R_n(i)$. This is done using a connected component criterion (figure 2). It allows to group different motion sections susceptible to be a part of the same region, or allows grouping the residual motion parts into one motion region. This propriety is useful to identify pedestrian who are not rigid and also useful in occultation of the moving object and other target.

$$D\left(f_m^{DC}, f_n^{DC}\right) = \sum_{i=1}^{X/8}\sum_{j=1}^{Y/8}\left|P(f_m^{DC}, I, i, j) - P(f_n^{DC}, I, i, j)\right| \quad (1)$$

Where $P(f_m^{DC}, I, i, j)$ is the DC coefficient of block (i, j). A scene change from f_m to f_n is declared if: (i) $D(f_m^{DC}, f_n^{DC})$ is the maximum within a symmetric sliding window and (ii) $D(f_m^{DC}, f_n^{DC})$ is 2-3 times the second largest maximum in the window.

Video shots may be associated with a key frame that best represents the shot and can later be used for the retrieval process. Let a shot represented by its first frame. Subsequent frames are then compared to the first frame, looking for a frame whose difference is above a given threshold T_s. If such a frame is found, it is considered as a key if it is followed by a continuous sequence of frames differing by at least T_s from the previous key frame. Choosing those frames of a video shot as key frames is based on the observation that consecutive frames are often almost identical. In addition, the shot is usually characterized by the first few frames, before the camera begins to zoom or close-up. So in our application it is a sufficient choice.

Motion Region Detection :

Then, all the moving objects must be accurately isolated from the background in order to be classified. Two methods are possible: temporal differencing (TD) and template correlation matching [Bre 97] [Bru 99] [Hua 83] [Kru 98] [Lip 98]. Both approaches have advantages and drawbacks. TD is impossible if there is a significant camera motion. It also fails if the target becomes occluded. On the other hand, the template correlation matching is not robust to changes in object size, orientation or even changing in light conditions. His use is most appropriate when the target size is small. So, the properties of these two methods are complementary. This is the motivation for combining TD and the notion of temporal consistency. The idea is to use TD to detect moving regions and apply temporal consistency algorithm to reduce misclassified motion regions.

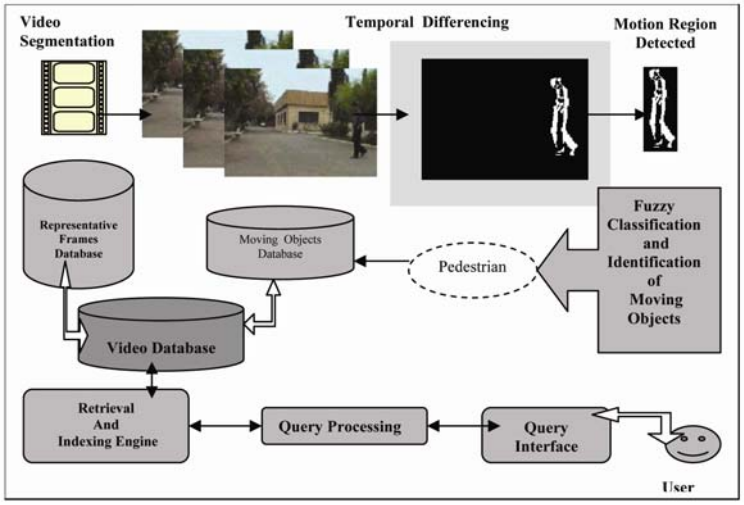

Fig. (1) : The video retrieval system overview

Video Segmentation:

The input data of the system consists of image sequences taken from outdoor video surveillance scenes. Video has both spatial and temporal dimensions and hence a good video index should capture the spatio temporal contents of the scene. In order to achieve this, the first step in video indexing is to decompose a video sequence into shots. Video shots may be associated with key or representative frames that best represent the shot. Several shot detection algorithms on compressed and uncompressed video are presented in [Yeo 95, 96] [Shn 96] [Bru 99] [Idr 97].

We propose to use a unified approach for scene change detection in motion JPEG and MPEG. This algorithm is based on the use of only DC coefficients. First we have to construct DC frame f_m^{DC} for every frame in the sequence. The DC coefficients in JPEG and I-frames in MPEG are obtained directly from each block. The DC coefficients for B- and P-frames are also estimated. The sum of the difference magnitude of the DC frames f_m^{DC} and f_n^{DC} is used as a measure of similarity between two frames.

sequence. Temporal features allow the user to specify queries that involve the exact positions and trajectories of the objects in the shot. The survey of what has been achieved on the content-based image retrieval in the past few years and what are the potential research directions can be found in [Bru 99] [Hab 99] [Fer 98] [Gud 95] [Idr 97] [Smo 94] [Sch 00] [Tiz 97].

Many content-based image search systems have been developed for various applications in order to extract intrinsic image features suitable of automatic indexing and retrieval. These features are used to reduce the complexity of image comparisons and to improve the organisation of image database. Unfortunately, automatic retrieval of suitable features is very hard; it is usually only feasible for retrieval systems that incorporate a high degree of domain-specific knowledge about the type of image contents to be retrieved. In unconstrained images, the set of known object classes is not available. Also, use of the image search systems varies greatly. The knowledge of the image content can be used to index specific images in the database for purposes of rapid retrieval [Ike 01] [Nib 93] [Sch 00]. In this context, we developed a system for retrieval and indexing telesurveillance MPEG videos in relation to the dynamic content of image sequences. It includes a robust fuzzy inference system to classify motion regions into pedestrians, vehicles and no-identified objects.

System Overview:

The system consists of five stages (figure 1). In the first stage (section 3), the digital video is segmented into elementary shots. In the second stage (section 4), all the moving objects are detected and segmented into motion regions. In the third stage (section 5), the principal idea is to exploit on one hand, the useful properties classification of fuzzy metrical classification in order to distinguish between types of motion regions, and on the other hand, the notion of temporal consistency in order to provide a robust classification against changes of objects appearance, occlusion, and cessation of object motion. In the fourth stage (section 6), once a motion region has been classified, it can be used as training template for the indexing and retrieval process.

Fuzzy Metrical System for Compressed Telesurveillance Databases Indexing

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Abstract:

This paper proposes a video retrieval system from compressed outdoor video surveillance databases. The aim is to extract moving objects from frames provided by MPEG video stream in order to classify them into predefined categories according to image-based properties, and then robustly index them. The principal idea is to combine between useful properties of metrical classification and the notion of temporal consistency. Fuzzy geometry classification is used in order to provide an efficient method to classify motion regions into three generic categories: pedestrian, vehicle and no identified object. The temporal consistency provides a robust classification to changes of objects appearance and occlusion of object motion. The classified motion regions are used as templates for metrical training algorithms and as keys for tree indexing technique.

Key words: Video database retrieval and indexing, Compressed video, Temporal consistency, Fuzzy geometry classification, Fuzzy inference system.

Introduction:

The large volume of images and videos pose a significant challenge for storage, retrieval and indexing the visual information from multimedia databases. Two approaches have been commonly used: a content indexing approach, where the index terms serve to encode the content of images; and a structural approach where images are represented as a hierarchy of regions, objects, and portions of objects. The content indexing approach is based on features such as colour, texture, shape and sketch extracted from an image, which essentially serve as the index. The structural approach is based on spatial relationships between objects or regions in a scene. In video indexing techniques using temporal features as keys, image sequences are indexed based on the motion properties of objects within the

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