

## قواعد النشر

مجلة دورية تنشرها إدارة النشر العلمي والترجمة بجامعة القصيم، وهي تهدف إلى إتاحة الفرصة للباحثين لنشر إنتاجهم العلمي وتقوم المجلة بنشر المواد الآتية:

- 1- بحث: يشمل على عمل المؤلف في مجال تخصصه، ويجب أن يحتوي على إضافة للمعرفة في مجاله.
- 2- مقالة استعراضية: تتضمن عرضاً نقدياً لبحوث سبق إجراؤها في مجال معين أو أجريت في خلال فترة زمنية محددة.
- 3- بحث مختصر.
- 4- نقد الكتب.
- 5- خطابات إلى المحرر، وملاحظات وردود، ونتائج أولية.

تقوم هيئة المحررين بالنظر في نشر المواد المعرفية ذات الصلة بذلك الفرع، وتقدم البحوث الأصلية، التي لم يسبق نشرها، بالإنجليزية أو بالعربية، وفي حال قبول البحث للنشر، لا يجوز نشره في أي منفذ نشر آخر ورقياً أو إلكترونياً، دون إذن كتابي من رئيس هيئة التحرير.

### تعليمات عامة

- 1- تقديم المواد: يقدم أصل البحث مخرجاً في صورته النهائية متضمناً الإشارة إلى أماكن الجداول والأشكال داخل المتن ومطبوعاً على هيئة صفحات مرقمة ترقيمياً متسلسلاً، مع ضرورة إرفاق قرص ممغنط مطبوع عليه البحث على برنامج Ms Word باستخدام النظام المتوافق مع IBM، وسيعتذر عن قبول أي بحث لا يلتزم مؤلفه بهذه التعليمات.
- 2- الملخصات: يرفق ملخصان بالعربية والإنجليزية للبحوث والمقالات الاستعراضية والبحوث المختصرة. على ألا يزيد عدد كلمات كل منهما على 200 كلمة، وعلى عمود واحد بعرض كتابة 13 سم.

- 3- لا بد من احتواء كل بحث على كلمات مفتاحية (Key Words) توضع أعلى الملخصين العربي والإنجليزي على ألا تزيد عن عشر كلمات.
- 4- الجداول والمواد التوضيحية: يجب أن تكون الجداول والرسومات واللوحات مناسبة لمساحة الصفح في صفحة المجلة (12 × 19 سم بالحواشي)، ويتم إعداد الأشكال الخطية على برامج الحاسب الآلي أو بالحبر الصيني الأسود على ورق كلك، ولا تقبل إلا أصول الأشكال. كما يجب أن تكون الخطوط واضحة ومحددة ومنظمة من حيث كثافة الحبر وتناسب سمكها مع حجم الرسم، ويراعى أن تكون الصور الفوتوغرافية (الضوئية) للملونة وغير الملونة مطبوعة على ورق لماع، أو محملة على برنامج (Adobe Photoshop)، مع كتابة عنوان لكل جدول، وتعليق لكل شكل وصورة، والإشارة إلى مصدر المادة إن كانت مقتبسة.

- 5- الاختصارات: يجب استخدام اختصارات عناوين الدوريات العلمية كما هو وارد في The World List of Scientific Periodicals وتستخدم الاختصارات المقننة دولياً مثل: سم، مم، م، كم، سم، ٢، مل، مجم، كجم... إلخ.

- 6- المراجع: يشار إلى المراجع داخل المتن بنظام الاسم والتاريخ، وتوضع المراجع جميعها في قائمة المراجع بنهاية المادة مرتبة هجائياً ومتبعة بنظام ترتيب البيانات الببليوجرافية التالي:

أ) يشار إلى الدوريات في المتن بنظام الاسم والتاريخ بين قوسين على مستوى السطر. أما في قائمة المراجع فيبدأ المرجع بذكر الاسم الأخير للمؤلف (اسم العائلة)، ثم الاسم الأول، ثم الأسماء الأخرى أو اختصاراتها بالخط الأسود. فعنوان البحث كاملاً بين قوسين " " فاسم الدورية مختصراً بنط مائل، فرقم المجلد، ثم رقم العدد بين قوسين، ثم سنة النشر بين قوسين، ثم أرقام الصفحات.

### مثال:

فقيهها، أنيس بن حمزة. "نمذجة تقطير خليط ذو نسبة تطاير عالية". مجلة جامعة الملك سعود (العلوم الهندسية)، المجلد ١٥، العدد (١)، (٢٠٠٣م)، ١٣- ٢٧.

ب) يشار إلى الكتب في المتن داخل قوسين بالاسم والتاريخ. أما في قائمة المراجع، فيكتب الاسم الأخير للمؤلف (اسم العائلة)، ثم الاسم الأول، ثم الأسماء الأخرى أو اختصاراتها بالخط الأسود. فعنوان الكتاب بنط مائل ثم بيان الطبعة. فمدينة النشر: ثم الناشر، ثم سنة النشر، ثم عدد صفحات الكتاب، إن وجدت.

### مثال:

المصري، وحيد عطية. مقدمة في هندسة العمليات الحيوية. الرياض: جامعة الملك سعود، ١٤٢٥هـ، ٥٠٠ ص.

ويجب عدم استخدام الاختصارات المرجعية مثل: المرجع نفسه. المرجع السابق... إلخ.

٧- الحواشي: تستخدم لتزويد القارئ بمعلومات توضيحية، ويشار إليها في المتن بأرقام مرتفعة عن السطر. وترقيم التعليقات متسلسلة داخل المتن. وفي حال الضرورة، يمكن الإشارة إلى مرجع داخل الحاشية عن طريق استخدام كتابة الاسم والتاريخ بين قوسين وبنفس طريقة استخدامها في المتن، وتوضع الحواشي أسفل الصفحة التي تخصها والتي ذكرت بها وتفصل بخط عن العمودين (المتن) وتكون الحواشي على سطر أو عمود واحد وليس عمودين.

- 8- المواد المنشورة في المجلة لا تعبر: بالضرورة، عن رأي جامعة القصيم.
- 9- المستلات: يعطى المؤلف (٢٠) عشرون نسخة مجانية من بحثه.
- 10- عناوين المراسلة

ترسل جميع مواد النشر والمكاتبات إلى :

رئيس التحرير

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المملكة العربية السعودية

١١- تصدر المجلة مرتين في العام.





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المجلد الثاني العدد (١)

# مجلة العلوم الزراعية والبيطرية

(محرم ١٤٣٠هـ)

(يناير ٢٠٠٩م)

المجلة العلمية لجامعة القصيم

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النشر العلمي والترجمة

جامعة القصيم

بريدية - ص.ب. ٦٦٦٦ - ٥١٤٥٢

# هيئة التحرير

## أعضاء هيئة تحرير المجلة

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أ.د. أسامة محمد محمود  
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## المحتويات

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# الإنتاج النباتي



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تم إجراء البحث في أحد البساتين الأهلية الواقعة في أبو غريب/بغداد للموسمين ٢٠٠٤ و ٢٠٠٥ على أشجار العنب صنف العباسي المرباة على العرائش (القمریات) لمعرفة تأثير التغذية الورقية بالزنك (صفر، ١٠٠، ٢٠٠ مجم/لتر) واليوريا (٠.٢٥٪، ٠.٥٠٪) في صفات النمو الخضري والثمري، وقد بينت النتائج ما يلي:

أدى رش الزنك واليوريا إلى تحسين المساحة الورقية وبعض الصفات الثمرية لاسيما المعاملة (٢٠٠ مجم/لتر زنك + ٠.٥٠٪ يوريا) إذ بلغت الزيادة الناتجة عنها ١٦.٠٠٪، ١١.٧٦٪، ١٥.٣٨٪، ١٠.٧٤ و ٦.٨٤٪ لصفات كمية الحاصل، وزن العنقود، عدد العناقيد، وزن ١٠٠ حبة والمساحة الورقية للموسم الأول بينما أعطت زيادة بلغت ٢١.٥٨٪، ١٤.٧٦٪، ٩.١٠٪، ٨.٨٣٪، ٦.٠٧٪ لنفس الصفات للموسم الثاني. كما أظهرت المعاملة ذاتها زيادة معنوية في نسبة النتروجين والفسفور والبوتاسيوم في الأوراق ولكلا الموسمين فيما أظهرت انخفاضاً معنوياً في نسبة السكريات الكلية.

: تسميد ورقي، زنك، يوريا، عنب، صنف عباسي.

لقد ذكر العنب في القرآن الكريم في عشر سور وثلاثة عشر آية وإن العنب التجاري يعود إلى الجنس *Vitis* وهو واحد من ١٤ جنس تابع للعائلة العنبية (السعيدى، ٢٠٠٠) *Vitaceae* التي تضم أكثر من ١٠٠٠ نوع وتنتشر بشكل كبير في المناطق الاستوائية والمعتدلة (الراوي، ٢٠٠٥). إن معظم الترب في المنطقة الوسطى من العراق تميل إلى القاعدية إذ يتراوح الـ PH لها بين (٧.٥ - ٨.٥) حسب محتواها من الكلس مما يجعل المغذيات تترسب على شكل مركبات معقدة وتصبح غير جاهزة للنبات (أبو ضاحي واليونس، ١٩٨٨). وتعتبر التغذية الورقية برش النباتات بمحاليل هذه المغذيات من أفضل الطرق الناجحة والسريعة لعلاج نقص العناصر (الدجيلي وآخرون، ١٩٩٤) و(الصحاف والدجيلي، ١٩٩٤) كما ذكر (Gravrilov, 1985) أن رش العنب بمخيلط من المنغنيز والزنك سبب زيادة في كمية الحاصل وأشار (Al-Sahaf, et al. 1993) أن رش اليوريا بتركيز ٠.٥٪ أعطى زيادة معنوية في الحاصل لبعض أصناف العنب. أما (الراوي، ٢٠٠٢) فقد وجد أن رش أشجار العنب صنف كمالي باليوريا قبل التزهير أدى إلى زيادة في كمية الحاصل وعدد العناقيد وزيادة في طول وقطر العنقود وكذلك زيادة في معدل وزن ٥٠ حبة. كما أشار (القوامي وآخرون، ٢٠٠٢) أن رش أشجار التين بمخيلط من الزنك والحديد والنايتروجين والجيرلين أدى إلى زيادة في معدل طول وقطر ووزن الثمار.

وذكر (Stover, 1999) أن رش أشجار التفاح بالبورون والزنك واليوريا قبل الإزهار أدت إلى زيادة في الحاصل وأشار (Righetti and Sanchez, 2005) أن إضافة اليوريا والبورون إلى أشجار التفاح أدت إلى زيادة في الأزهار. لقد اجري هذا البحث لدراسة استجابة شجيرات العنب صنف عباسي لمستويات من التسميد الورقي بالزنك واليوريا بغية تحسين مواصفات النمو الخضري والثمري وزيادة الإنتاج.

أجري البحث في أحد بساتين العنب الأهلية في بغداد / أبو غريب للموسمين ٢٠٠٤ و ٢٠٠٥م لدراسة تأثير الرش بالزنك واليوريا كل على انفراد والتوليفات بينهما في بعض صفات النمو الخضري والثمري لصنف العنب "عباسي". تم اختيار شجيرات متجانسة النمو قدر الإمكان بعمر ٩ سنوات مزروعة بمسافات (٣×٤م) وأخذت عينات من تربة البستان بأعماق ٠ - ٣٠ ، ٣٠ - ٦٠ ، ٦٠ - ٩٠ سم لدراسة الصفات الفيزيائية والكيميائية للتربة (الجدول رقم ١) وقد تم تقليم الشجيرات في شهر كانون الثاني إذ ترك عدد ثابت من العيون وبمعدل ١٠ قصبات لكل شجرة وبطول ١٠ عين/قصبه. نفذت تجربة عاملية تم فيها دراسة التسميد الورقي بعاملين الاول الزنك ومستوياته (صفر، ١٠٠ ، ٢٠٠ مجم/ لتر) والثاني اليوريا ومستوياته (صفر ، ٢٥.٠٪ ، ٥٠.٠٪) مع دراسة الفعل المتبادل. وقد وزعت المعاملات وفق تصميم القطاعات العشوائية الكاملة (RCBD) وبثلاثة مكررات لكل معاملة وبواقع شجيرة واحدة لكل مكرر. وقد تم

الرش بالمعاملات المذكورة سابقاً بعد إضافة المادة الناشرة (Tween 20) وبتركيز (١ مل/لتر) إلى محلول الرش لتقليل الشد السطحي لجزيئات الماء لغرض إحداث البلل الجيد للأشجار المعاملة. وتم الرش في الصباح الباكر وقبل موعد التزهير بعشرة ايام ثم اعيد الرش مرة ثانية بعد العقد بعشرة ايام . ودرست المؤشرات التالية :

١- المساحة الورقية للشجيرة (م<sup>٢</sup>/شجيرة).

٢- محتوى الأوراق من N,P,K حيث أخذت الأوراق في مرحلة الاتساع الكامل في شهر حزيران وقدر

النيتروجين بوساطة جهاز مايكر كلدال Microkjeldal والفسفور بالمطياف الضوئي Spectrophotometer والبوتاسيوم بوساطة جهاز Flamephotometer .

٣- متوسط الحاصل الكلي /كجم.

٤- متوسط وزن العنقود /جم.

٥- متوسط عدد العناقيد /شجرة.

٦- متوسط وزن ١٠٠ حبة.

٧- النسبة المئوية للسكريات الكلية.

٨- النسبة المئوية للحموضة الكلية.

وقد حللت النتائج حسب التصميم المتبع وقورنت النتائج وفق أقل فرق معنوي LSD عند مستوى

معنوية ٠,٠٥ (الراوي وعبد العزيز، ١٩٨٨).

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يظهر الجدول رقم (٣) أن المعاملة المشتركة (زنك +٢٠٠٪ يوريا) قد أعطت أعلى كمية حاصل بلغت ١٥.٦٦ و ١٧.٠٧ كجم/ شجرة للموسمين على التوالي وتفوقت هي ومعاملة (زنك +١٠٠٪ يوريا) على باقي المعاملات وقد أعطت معاملة المقارنة أقل كمية حاصل للموسمين بلغت ١٣.٥٠ و ١٤.٠٤ كجم/ شجرة. كما سجلت المعاملة ذاتها أعلى معدل لوزن العنقود بلغ ٤١٥.١ جم للموسم الأول وتفوقت معنوياً على باقي المعاملات بينما أعطت ٤٣٦.٩ جم للموسم الثاني وتفوقت على باقي المعاملات باستثناء معاملة (زنك +١٠٠٪ يوريا) التي لم تختلف عنها معنوياً بينما أعطت معاملة المقارنة أقل معدل لوزن العنقود للموسمين حيث سجل ٣٧١.٤ و ٣٨٠.٧ جم. أما عدد العناقيد/ شجرة فقد أعطت المعاملة المشتركة (زنك +٢٠٠٪ يوريا) أعلى معدل لعدد العناقيد بلغ ٣٩.٨٢ و ٤٠.٥٢ لموسمي البحث وتفوق معنوياً هو ومعاملة (زنك +١٠٠٪ يوريا) على باقي المعاملات فيما أعطت معاملة المقارنة أقل معدل لعدد العناقيد للموسمين ٣٤.٥١ و ٣٧.١٤. كذلك فإن معدل وزن ١٠٠ حبة سار بنفس الاتجاه حيث أعطت معاملة (زنك +٢٠٠٪ يوريا) أعلى معدل للموسمين ٣٩٦.٧ و ٣٩٣.٠٠ جم للموسمين على التوالي وتفوق معنوياً هو ومعاملة (زنك +١٠٠٪ يوريا) على باقي المعاملات باستثناء المعاملة (زنك +٢٠٠٪ يوريا) فيما أعطت معاملة المقارنة أقل معدل للموسمين ٣٥٨.٢ و ٣٦١.١ جم.

إن الزيادة في كمية الحاصل ووزن وعدد العناقيد قد تعود إلى أن الرش بالمغذيات قد أدى إلى تحسين نمو الأشجار وزاد من كفاءة الأوراق في صنع الغذاء كما أن للمغذيات دور مهم في تحسين بناء الأوعية الناقلة وتوجيه



السبب إلى زيادة كمية الحاصل لنفس المعاملة حيث يرافق زيادة الحاصل نقصان في كمية السكريات الكلية وهذا يوافق ما ذكره (الراوي، ١٩٩٤) الذي ذكر أن الحاصل العالي قد يرافقه نقصان في نسبة السكريات والمواد الصلبة الذائبة الكلية. بينما لم تكن هناك فروق معنوية في صفة الحموضة الكلية بين المعاملات وللموسمين. تظهر نتائج هذه الدراسة أنه بالإمكان زيادة حاصل العنب صنف العباسي وتحسين نوعية الحبات وذلك بالرش بالزنك بتركيز ٢٠٠ مجم/لتر واليوريا بتركيز ٠.٥٠٪.

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**Effect of Foliar Application With Some Level of Zinc and Urea on Yield and Fruit Characters in Grape *Vitis vinifera* L. CV. Al-Abbasi**

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**Abstract.** This study was conducted in a private orchard at Abu Ghraib/Baghdad during the growing seasons 2004 and 2005 on grape CV. abbasi trained on wires by using pergolas system to investigate the influences of foliar application with Zinc and Urea in growth and yield and its quality. Zinc was used at (0\100\200mg\L) while Urea was used at (0\0.25%\0.50%). Data indicated that foliar spray with Zinc and Urea improved the leaf area, yield and some fruit characters specially the treatment with 200 mg/L Zinc and 0.50% and the increment was 16%, 11.76%, 15.38%, 10.74% and 6.84% for the first season and 21.58%, 14.76%, 9.10%, 8.83% and 6.07% for the second season for the characters : the total yield, bunch weight, the number of bunch, the weight of 100 berries and leaf area. The same treatment significantly increased the percentage of Nitrogen, Phosphorus and Potassium in the leaves, while this treatment significantly reduced the total sugar.

**Key words:** Foliar application, zn, urea, grape, abbasi, fruit characters



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# **ANIMAL PRODUCTION**



## **Carcass, Tissues Composition and Meat Quality Traits in crossed V-Line with Saudi Gabali Rabbits**

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**Abstract.** A five-year crossbreeding project involving Spanish maternal line called V-line (V) and Saudi Gabali (S) rabbits was carried out to produce 14 genetic groups of V, S,  $\frac{1}{2}V\frac{1}{2}S$ ,  $\frac{1}{2}S\frac{1}{2}V$ ,  $\frac{3}{4}V\frac{1}{4}S$ ,  $\frac{3}{4}S\frac{1}{4}V$ ,  $(\frac{1}{2}V\frac{1}{2}S)^2$ ,  $(\frac{1}{2}S\frac{1}{2}V)^2$ ,  $(\frac{3}{4}V\frac{1}{4}S)^2$ ,  $(\frac{3}{4}S\frac{1}{4}V)^2$ ,  $((\frac{3}{4}V\frac{1}{4}S)^2)^2$ ,  $((\frac{3}{4}S\frac{1}{4}V)^2)^2$ , Saudi 2 (synthetic maternal line), and Saudi 3 (synthetic paternal line). A total number of 2770 rabbits produced by 91 sires and 402 dams were used to evaluate carcass components, tissue composition and meat quality traits. A generalized least square procedure was used to estimate additive and heterotic effects (direct, maternal, and grand-maternal). The estimates of direct additive effects were significant and in favour of V line rabbits for the majority of the traits studied, ranging from 3.8 to 9.0% for slaughter and edible carcass components, 3.4 to 10% for non-edible traits, -3.1 to 9.8 % for tissues compositions, and -14.9 to 2.5 % for meat quality traits. Maternal additive effects were significantly in favour of V line by 1.66% for meat ether extract (11.1% relative to the average of the V line and Gabali as purebreds). Grand-maternal additive effects were not significant in most traits studied except for dry matter and ash contents in meat. The effect of the V line was found higher than that of the Gabali by 0.5% and 1.39%, respectively (0.7% and 15.4% of the respective averages of the pure breeds). Heterosis estimated for non-edible traits were mostly positive and only significant for head weight (direct and grand-maternal heterosis), fur weight (grand-maternal heterosis), lung weight (maternal and grand-maternal heterosis) and visceral weight (maternal and grand-maternal heterosis). The estimates were small relative to the average of purebreds reaching 6.4% as maximum value. Estimates of direct, maternal and grand-maternal heterosis for meat weight were found to be consistent and positive (3.9, 4.5 and 5%, respectively) associated with significant direct heterosis for fat weight (12.2%), maternal heterosis for meat bone ratio (4.5%), and maternal and grand-maternal heterosis for dry matter in meat. The estimates of direct heterosis for protein content in meat were significantly positive (1.4%), but the estimates for grand-maternal heterosis were significantly negative (-2.1%). For fat content in meat, the estimates of direct (-8.3%) and maternal heterosis (-11.9%) were significant, while for ash content, the estimates for maternal (23.7%) and grand-maternal heterosis (30.1%) were significantly positive.

**Keywords:** Rabbits, crossbreeding, carcass and meat quality, additive effects, heterosis.

## Introduction

Reports on genetic analysis for carcass components and meat quality for rabbits raised in hot climates are scarce. Since 2000, a co-operative rabbit project was established between Saudi Arabia and Spain. The V-line rabbits used in this project were imported from Universidad Politécnica de Valencia in Spain to develop new lines of meat rabbits convenient for hot climate (Khalil *et al.*, 2007). Line V was, then crossed with a local breed named Saudi Gabali. Currently, these synthetic lines have reached F<sub>7</sub> progeny. In this project, genetic analyses for some traits such as litter size, lactation traits, feeding traits and semen parameters have been genetically evaluated (Khalil *et al.*, 2004, 2005). Carcass components have not been studied. The objective of the present study was mainly to evaluate, genetically, carcass and meat quality traits as influenced by the additive and heterotic effects (direct, maternal, and grand-maternal).

## Material and Methods

### Animals and crossbreeding plan

Five-years crossbreeding project involving a desert Saudi Gabali (S) and a Spanish V-line (V) rabbits was started in September 2000 in the experimental rabbitry, College of Agriculture and Veterinary Medicine, Al-Qassim University in Saudi Arabia. Eighty pedigreed does and sixteen pedigreed bucks of V-line rabbits were imported from Universidad Politécnica de Valencia (Spain) in September 2000. V-line is a maternal rabbit line selected for number of young weaned per litter for 21 generations (Estany *et al.*, 1989), while Saudi Gabali is a Saudi breed raised under the desert conditions, especially in Najd area, and rabbits of this breed are characterized by litter size of 6-8 young, mature body weight of 3.2-3.8 kg and the ability to survive and adapt to produce and reproduce under hot environment. Details of housing, feeding, procedures and crossbreeding plan used in the project to form new synthetic lines were described by Khalil *et al.* (2007). This crossbreeding plan permitted simultaneous production of 14 genetic groups of V, S,  $\frac{1}{2}V\frac{1}{2}S$ ,  $\frac{1}{2}S\frac{1}{2}V$ ,  $\frac{3}{4}V\frac{1}{4}S$ ,  $\frac{3}{4}S\frac{1}{4}V$ ,  $(\frac{1}{2}V\frac{1}{2}S)^2$ ,  $(\frac{1}{2}S\frac{1}{2}V)^2$ ,  $(\frac{3}{4}V\frac{1}{4}S)^2$ ,  $(\frac{3}{4}S\frac{1}{4}V)^2$ ,  $((\frac{3}{4}V\frac{1}{4}S)^2)^2$ ,  $((\frac{3}{4}S\frac{1}{4}V)^2)^2$ , **Saudi 2** [synthetic maternal line with a genetic structure of  $((\frac{3}{4}V\frac{1}{4}S)^2)^2$  interse mated] and **Saudi 3** [synthetic paternal line with a genetic structure of  $((\frac{3}{4}S\frac{1}{4}V)^2)^2$  interse mated]. The bucks were randomly assigned to mate the does naturally with the restriction to avoid the mating of animals with common grandparents. A total number of 2770 rabbits produced by 91 sires and 402 dams were slaughtered.

### Data set

Data obtained in this study have been recorded from November 2000 until July 2005. At 12 weeks of age, rabbits were slaughtered and hot carcasses were weighed and dressing percentages were calculated. The head, fur, offal (representing heart + liver + kidneys) and viscera were also weighed. For lean composition traits, the right half of the carcass was separated into lean, fat and bone. Lean of each half was separated and prepared for chemical analysis. Dry matter (using an air-evacuated oven for 16 h), crude protein (N x 6.25), ether extract and ash in the lean were determined according to the A.O.A.C. (1990).

### Statistical analysis and estimation of crossbreeding genetic parameters

The animal model (in matrix notation) used for analysing carcass and meat quality traits was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_a\mathbf{u}_a + \mathbf{Z}_c\mathbf{u}_c + \mathbf{e}$$

Where,  $\mathbf{y}$  = vector of observed trait for the slaughtered rabbit,  $\mathbf{b}$  = vector of fixed effects of genetic group of slaughtered rabbit (14 levels), and year-season of birth of the slaughtered rabbit (20 levels), sex, parity order of the doe (five levels), and litter size at birth (9 levels);  $\mathbf{u}_a$  = vector of random additive effect of the individual rabbit,  $\mathbf{u}_c$  = vector of random effects of the litter in which the animal was born (non-additive litter common effect);  $\mathbf{X}$ ,  $\mathbf{Z}_a$  and  $\mathbf{Z}_c$  = incidence matrices relating the records to the fixed effects, additive genetic effects, and common litter environment, respectively; and  $\mathbf{e}$  = vector of random residual effects.

$\text{Var}(\mathbf{u}_a) = \mathbf{A}\sigma_a^2$ , where  $\mathbf{A}$  is the numerator relationship matrix,  $\text{Var}(\mathbf{u}_c) = \mathbf{I}\sigma_c^2$  and  $\text{Var}(\mathbf{e}) = \mathbf{I}\sigma_e^2$ . Variance components of the random effects were estimated using MTDFREML software of Boldmann *et al.* (1995). Heritability estimates and common litter effects for different traits used in this study were given in Al-Saeef *et al.* (2007). These estimates were used to solve the corresponding mixed model equations, obtaining solutions for the genetic group means and their error variance-covariance matrix, using the PEST program (Groeneveld, 2006). A procedure of generalized least squares (GLS) was applied to get the estimates of the crossbreeding genetic parameters using the following linear model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{e}, \quad \text{Var}(\mathbf{y}) = \mathbf{V}$$

Where,  $\mathbf{y}$  was the vector of estimated groups means, given as difference to the second genetic group (Saudi Gabali);  $\mathbf{X}$  was a incidence matrix relating  $\mathbf{y}$  to  $\mathbf{b}$  (Dickerson, 1992),  $\mathbf{b}$  was the vector of estimable crossbreeding genetic parameters,  $\mathbf{e}$  was the vector of residual effects, and  $\mathbf{V}$  was the error variance-covariance matrix of  $\mathbf{y}$ . The components of  $\mathbf{b}$  were the difference between direct additive effects between V and G ( $D=D_V-D_S$ ), the difference between maternal additive effects between V and G ( $M=M_V-M_S$ ), the difference between grand-maternal additive effects between V and G ( $GM=GM_V-GM_S$ ), direct ( $H^D$ ), maternal ( $H^M$ ), and grand-maternal ( $H^{GM}$ ) heterosis.

## Results and Discussion

### Direct, maternal and grand-maternal additive effects

The estimates of direct additive effects were significant for the majority of traits (Table 1). In general, the effects for V line were higher than the effects for Saudi Gabali, but the effects for dry matter and ash contents in the meat were higher for Saudi Gabali. The percentages of these effects ranged from 3.8 to 9.0% for slaughter and edible carcass components, 3.4 to 10% for non-edible carcass components, -3.1 to 9.8 % for tissues compositions, and -14.9 to 2.5 % for meat quality traits.

The maternal additive effect was significant only for ether extract content in the meat. The difference between V line and Saudi Gabali was 1.66% (11.1% relative to the average of V line and Saudi Gabali as purebreds, Table 1). Piles *et al.* (2004) found that maternal genetic effects were not significant for dressing out percentage, drip loss weight and chilled carcass weight. The grand-maternal additive effects were only significant for some meat quality traits of dry matter content and ash contents in meat (Table 1). In both traits, the effects for V line was higher than the effect of Saudi Gabali by 0.5% and 1.39%, respectively (0.7% and 15.4% of the respective averages of the pure breeds).

**Table (1). Estimates of direct, maternal and grand-maternal additive effects (differences 'V-S' between V and Saudi Gabali line effects) and their standard errors ( $\pm$ SE) for carcass and meat quality traits**

Trait <sup>1</sup>	Direct additive effects		Maternal additive effects		Grand-maternal additive effects	
	Estimate $\pm$ SE	% <sup>1</sup>	Estimate $\pm$ SE	% <sup>1</sup>	Estimate $\pm$ SE	% <sup>2</sup>
PSW, g	123 $\pm$ 45*	5.3	12 $\pm$ 44	0.5	-1 $\pm$ 36	-0.0
Edible carcass components:						
HCW, g	120 $\pm$ 30*	9.0	12 $\pm$ 29	0.9	6 $\pm$ 24	0.5
DP, %	2.09 $\pm$ 0.43*	3.8	0.26 $\pm$ 0.41	0.0	0.27 $\pm$ 0.34	0.0
OW, g	4.19 $\pm$ 3.24	4.4	-0.28 $\pm$ 3.14	-0.3	2.11 $\pm$ 2.60	2.2
Non-edible carcass components:						
HW, g	12.09 $\pm$ 3.78*	5.5	2.63 $\pm$ 3.66	1.2	-1.14 $\pm$ 3.02	-0.5
FURW, g	7.54 $\pm$ 5.99	3.4	7.02 $\pm$ 5.83	3.1	1.52 $\pm$ 4.87	0.7
LW, g	9.66 $\pm$ 2.00*	10.0	-2.05 $\pm$ 1.97	-2.1	-0.05 $\pm$ 1.67	-0.0
VW, g	29.2 $\pm$ 9.9*	7.7	-16.2 $\pm$ 9.6	-4.3	5.4 $\pm$ 7.9	1.4
Tissues composition in the carcass:						
MW, g	61 $\pm$ 22*	6.2	18 $\pm$ 21	1.8	2 $\pm$ 18	0.0
BW, g	26 $\pm$ 8*	9.8	-1 $\pm$ 8	-0.4	-0 $\pm$ 7	-0.0
FW, g	0.25 $\pm$ 2.29	1.0	0.78 $\pm$ 2.21	3.1	-0.75 $\pm$ 1.84	-3.0
MBR	-0.12 $\pm$ 0.12	-3.1	0.06 $\pm$ 0.12	1.6	0.02 $\pm$ 0.10	0.5
Meat quality traits:						
DM, %	-0.99 $\pm$ 0.30*	-3.3	-0.35 $\pm$ 0.29	-1.2	0.50 $\pm$ 0.24*	1.7
CP, % <sup>++</sup>	1.87 $\pm$ 0.91*	2.5	-1.34 $\pm$ 0.90	-1.8	-0.65 $\pm$ 0.75	-0.9
EE, % <sup>++</sup>	-0.06 $\pm$ 0.87	-4.0	1.66 $\pm$ 0.87*	11.1	-0.80 $\pm$ 0.74	-5.3
Ash, % <sup>++</sup>	-1.34 $\pm$ 0.60*	-14.9	-0.38 $\pm$ 0.58	-4.2	1.39 $\pm$ 0.49*	15.4

<sup>1</sup> PSW= Pre-slaughter weight, HCW= Hot carcass weight, DP= Dressing percent, OW= Offal weight, HW= Head weight, FURW= Fur weight, LW= Lung weight, VW= Viscera weight, MW= Meat weight, BW= Bone weight, FW= Fat weight, MBR= Meat to bone ratio, DM= Dry matter, CP= Crude protein, EE= Ether extract; <sup>2</sup>Percentage of the difference referred to the average of the values for V line and Saudi Gabali breed; \*significant at  $\alpha=0.05$ .

### Direct, maternal and grand-maternal heterosis

Estimates of direct, maternal and grand-maternal heterosis are shown in Table 2. For edible carcass components, estimates of direct heterosis were not significant, but for maternal heterosis the estimates were positively significant for hot carcass weight and dressing percentage. However, the relative importance of these effects were practically negligible (2.4 and 1.3%). For grand-maternal heterosis, the estimates were significant for pre-slaughter weight (96 g, 4.0%) and dressing percentage (-0.70% and -1.3% relative to purebreds). The opposite signs for pre-slaughter weight and for dressing percentage explained the non significance heterosis obtained for hot carcass weight (Table 2). All these results showed that the chance to improve edible components of the carcass by crossing could be limited. However, most estimates of heterosis obtained from experiments in USA, Egypt and France (Lukefahr *et al.*, 1983; Brun and Ouhayoun, 1989; Afifi *et al.*, 1994; Khalil and Afifi, 2000) indicated that crossbreeding in rabbits was associated with little improvement in the carcass performance.

**Table (2). Estimates of direct, maternal and grand-maternal heterosis and their standard errors ( $\pm$ SE) for carcass and meat quality traits.**

Trait <sup>1</sup>	Direct heterosis		Maternal heterosis		Grand-maternal heterosis	
	Estimate $\pm$ SE	% <sup>2</sup>	Estimate $\pm$ SE	% <sup>2</sup>	Estimate $\pm$ SE	% <sup>2</sup>
PSW, g	42 $\pm$ 23	1.8	31 $\pm$ 22	1.3	96 $\pm$ 28*	4.0
Edible carcass components:						
HCW, g	28 $\pm$ 15	2.1	32 $\pm$ 15*	2.4	30 $\pm$ 18	2.3
DP, %	0.39 $\pm$ 0.21	0.7	0.71 $\pm$ 0.21*	1.3	-0.70 $\pm$ 0.26*	-1.3
OW, g	3.07 $\pm$ 1.62	3.2	0.51 $\pm$ 1.60	0.5	3.89 $\pm$ 2.00	4.1
Non-edible traits:						
HW, g	5.13 $\pm$ 1.88*	2.4	2.98 $\pm$ 1.87	1.4	5.81 $\pm$ 2.32*	2.7
FURW, g	5.13 $\pm$ 3.03	2.2	1.38 $\pm$ 2.98	0.6	11.80 $\pm$ 3.73*	5.3
LW, g	0.61 $\pm$ 1.06	0.6	4.55 $\pm$ 1.03*	4.6	4.69 $\pm$ 1.28*	4.7
VW, g	-0.9 $\pm$ 4.9	-0.0	18.3 $\pm$ 4.9*	4.8	24.4 $\pm$ 6.1*	6.4
Tissues composition in the carcass:						
MW, g	38 $\pm$ 11*	3.9	44 $\pm$ 11*	4.5	49 $\pm$ 14*	5.0
BW, g	3 $\pm$ 4	1.1	-1 $\pm$ 4	-0.4	7 $\pm$ 5	2.6
FW, g	3.04 $\pm$ 1.14*	12.2	-1.24 $\pm$ 1.13	-5.0	1.73 $\pm$ 1.41	6.9
MBR	0.07 $\pm$ 0.06	1.8	0.17 $\pm$ 0.06*	4.5	0.06 $\pm$ 0.07	1.6
Meat quality traits:						
DM, %	0.06 $\pm$ 0.15	0.2	0.50 $\pm$ 0.15*	1.7	0.79 $\pm$ 0.18*	2.6
CP, %	1.04 $\pm$ 0.45*	1.4	-0.18 $\pm$ 0.46	-0.2	-1.59 $\pm$ 0.59*	-2.1
EE, %	-1.25 $\pm$ 0.44*	-8.3	-1.79 $\pm$ 0.45*	-11.9	-0.83 $\pm$ 0.58	-5.5
Ash, %	0.37 $\pm$ 0.029	4.1	2.13 $\pm$ 0.29*	23.7	2.71 $\pm$ 0.37*	30.1

<sup>1</sup> See table 1; <sup>2</sup> Percentage of the heterosis referred to the average of the values for V line and Saudi Gabali breed. NS= non-significant, \*significant at  $\alpha=0.05$ .

Concerning non-edible traits, heterosis estimates were mostly positive and only significant for head weight (direct and grand-maternal heterosis), fur weight (grand-maternal heterosis), lung weight (maternal and grand-maternal heterosis) and viscera weight (maternal and grand-maternal heterosis). These estimates relative to the average of the purebreds were small reaching 6.4% as maximum value. Also, the positive signs of heterosis obtained for these traits were economically unfavorable. In reference to heterosis for tissue composition of the carcass, the estimates of direct, maternal and grand-maternal heterosis for meat weight were found to be consistent and positive (3.9, 4.5 and 5%, respectively). Estimates of direct heterosis for fat weight (12.2%) and the maternal heterosis for meat bone ratio (4.5%) were significant (Table 2). Heterosis for meat quality traits seemed more important, increasing a little in dry matter content since maternal and grand-maternal heterosis were significant. In average, the estimates of direct heterosis for protein content in meat were significantly positive (1.4%), but the estimates for grand-maternal heterosis were significantly negative (-2.1%). For fat content in meat, the estimates of direct (-8.3%) and maternal heterosis (-11.9%) were significant, while for ash content the estimates for maternal (23.7%) and grand-maternal heterosis (30.1%) were significantly positive.

## Conclusions

1. Differences in direct additive effects were frequent for the studied traits and generally in favor of V line, while maternal and grand maternal additive effects were less important and only appeared to be significant in some meat quality traits.
2. Heterosis found in this experiment are of small importance, particularly for edible carcass components and non edible traits. For the traits related to the tissue compositions, the importance was consistent, and for meat quality traits the importance was considerable.

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(قدم للنشر في ٢٠٠٩/٣/٧ م؛ وقبل للنشر في ٢٠٠٩/٥/٩ م)

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## **Effect of Urea Molasses Based Supplementation on the Overall Feed Efficiency and Reproductive Performance of Dairy Cattle Under Grazing Management**

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**Abstract.** Importance of a complementary combination of concentrates and forages to increase the production of milk has been well documented. Recently increasing the rumen degradable protein (RDP) content in the diet to allow a better utilization of forage energy has been debated. In this on farm experiment, 54 multiparous Sahiwal cows in late pregnancy were fed with the following: 1) Conventional concentrate 2) Urea supplement and 3) Urea and fish meal supplement. All cows were managed in free grazing system and taken to the milking parlor twice daily for milking. Cows were fed during milking and the intake was recorded. Milk yield was recorded for each milking, and samples were analyzed for composition. All the reproductive events were recorded. The results suggest that partial substitution of copra meal with urea in molasses based concentrate would reduce the cost of feeding while increasing the milk yield and persistency and enhance the reproductive performance in cows maintained on free grazing condition.

**Keywords:** Urea, fish meal, cows, estrus detection, rumen protection.

## Introduction

Dairying becomes expensive and ruminant production systems in many parts of the world has to battle for the existence. Increased cost of production as the results of lack of quality feed ingredients has aggravated the livestock industry. On the other hand, ever increasing demand for livestock products and intensive breeding programs lead the industry with improved genetic potentiality of the livestock population. This situation necessitates farmers to compromise between the cost and the quality of feed. Consequently cost efficiency become major concern and supplementation with non conventional feed sources would be one of the alternatives to curb the cost of production. Urea molasses based supplements have been shown to increase the feed efficiency of low quality roughages and very successful in many countries (Taiwo, A.A., *et al.*, 1992; Khanal, R.C., *et al.*, 1997; Uthayathas, S., *et al.*, 1998, 1999; Waruiru, R.M, 2004). Urea may be an economical means of providing some of the required nitrogen to ruminants. It has been shown that increased availability of ammonia to the micro organism in the rumen increase the feed intake and improves the efficiency of microbial protein synthesis (Charbonneau, E., *et al.*, 2007). Molasses is one of the by products obtained from sugar industry that has large amount of soluble carbohydrate. Supplying high quality proteins containing essential amino acids in the form of rumen protected protein has been shown to increase the performance of cows (Trínáctý, J., *et al.*, 2006). Fish meal is a very good source of rumen protected protein. Fish meal contains many essential amino acids and shown to enhance milk production in high yielding dairy cows (Korhonen, M. *et al.*, 2002). Dairy cows producing less than 10 liters of milk per day may be supported by the microbial protein synthesis and rumen degradable proteins, such as in the form of urea play a vital role in those production systems (Taiwo, A.A., *et al.*, 1992; Mathur, O.P., *et al.*, 1994; Noftsker, S. and St-Pierre, N. R., 2003).

Urea molasses based supplements have a potential feeding value however the role of it on the reproductive parameters under grazing management in tropical environment has not been established. Therefore, current study was conducted to evaluate the effect of urea molasses based supplements for dairy cows under grazing condition in an on farm experiment. The results revealed that inclusion of fishmeal by substituting copra meal didn't produced expected benefit due to reduced palatability to the supplement. Here we show that substitution of copra meal with urea and molasses improves nitrogen intake, milk yield and persistency and reproductive performance while lowers the cost of production.

## Material and Methods

### Cows, diets, and experimental design

Fifty four multiparous Sahiwal cows in late pregnancy [ $2.2 \pm 0.3$  parity;  $369 \pm 23$  kg of Body weight (BW);  $5.14 \pm 35$  kg/d of milk yield in previous lactation (mean  $\pm$  SD)] were used in a randomized complete block design for the entire lactation. Milk yield of the previous lactation was used as a source of variation to group the cows to reduce the experimental error. Cows were kept in a free grazing condition on signal grass (*Brachiaria brizantha*) under coconut plantation. All cows were taken to the milking parlor twice a day at 5:30 a.m. and 3:00 p.m. for milking and the test supplements were fed individually during milking. Treatments were 1) Conventional concentrate as control 2) Urea supplement and 3) Urea and fish meal supplement (Table 1). This experiment was designed to test a practical feeding program and therefore, higher nitrogen with minimum cost was provided as urea supplement.

**Table (1). Ingredients of experimental diets (Fresh matter basis).**

Ingredients	Treatment		
	<sup>1</sup> Control (g/kg)	Urea (g/kg)	Fish meal urea (g/kg)
Molasses	375	375	375
Urea	--	40	40
<sup>2</sup> Fish meal	--	--	30
Copra meal	200	100	50
Rice bran	375	435	455
Salt	30	30	30
<sup>3</sup> Vitamins and minerals	20	20	20

<sup>1</sup>Conventional concentrate mixture used in the farm. All the cows in this experiment were on this supplement during the previous lactation. There was no supplementation during the dry period.

<sup>2</sup>Commercially available fish meal imported from Denmark is purchased from The Colombo Forage Stores Ltd, Colombo, Sri Lanka,

<sup>3</sup>Mineral composition (Dry matter basis): Ca = 11.05%; P = 7.36%; Na = 13.07%; Cl = 20.52%; Mg = 3.68%; K = 0.18%; S = 0.11%; Cu = 801 mg/kg; Mn = 2,670 mg/kg; Zn = 4,009 mg/kg; Fe = 4,235 mg/kg; Co = 29.46 mg/kg; I = 80.09 mg/kg; Se = 16.57 mg/kg; vitamin A = 360,000 UI/kg; vitamin D = 122,000 UI/kg; vitamin E = 1,215 UI/kg.

### Measurements and sampling

Individual feed intake was recorded daily and orts were sampled each following day. Subsamples were pooled by treatments per period and kept at  $-20^{\circ}\text{C}$  for chemical analysis. Feed were analyzed for proximate composition (AOAC, 1990). Grass intake was determined by measuring the sward height before and after 24 hours of grazing. Milk yield was recorded for each milkings, and milk was sampled fortnightly for further composition analyses. Milk samples were analyzed for protein content using Kjeldhal method and fat content using Gerber method (Kleyn, D.H., *et al.*, 2001). After three months of introducing the test feeds, blood were sampled to analysis the blood urea nitrogen (Uthayathas, S., *et al.*, 1999). Visual observations of estrus were made twice daily and milk samples of cows showing signs were assayed for progesterone level for confirmation. The milk fat was removed by centrifugation and skim milk samples analysed for progesterone. Progesterone was estimated by the radioimmunoassay RIA method using DPC kits, supplied by IAEA (FAO, 1993). Cows manifesting standing estrus were inseminated commencing 60 days postpartum. Progesterone assay was also used to diagnose the initial stage of pregnancy however, confirmed by palpation of the uterine tract at 40 or so days following breeding. All the reproductive events such as dates of first standing estrus, insemination, pregnancy test results, dry period and calving were recorded for individual cows and the parameters were calculated. Number of days to first standing heat, Number of services per conception, days open and length of calving interval were tabulated.

### Statistical analysis

Milk yield and composition, dry matter intake and reproductive parameters were averaged for each cow. Data were analyzed in two-way ANOVA using the SAS (SAS Institute, 1985). Duncan's new multiple range test was used to separate the means. Reproductive parameters were analyzed in a non-parametric test because of the non-normal distribution of data. Results were declared significant for  $P < 0.05$ .

## Results

Proximate composition and feed and nutrient intake are presented in Table (2). Intake of supplementary diets increased with increasing level of copra meal which might be due to the palatability factor. There was a significant difference in N intake among concentrates.

Milk yield and 4% fat corrected milk yield are presented in Table (3).

**Table (2). Food intake and proximate composition of experimental diets.**

<i>Proximate composition</i>	<i>Control</i>	<i>Urea</i>	<i>Fish meal urea</i>	<i>Grass</i>
Dry matter (%)	857.6 $\pm$ 7.4	862.4 $\pm$ 8.3	861.3 $\pm$ 6.4	216.7 $\pm$ 12.6
*Crude protein	<sup>a</sup> 108.3 $\pm$ 3.2	<sup>b</sup> 206.3 $\pm$ 2.4	<sup>b</sup> 219.4 $\pm$ 2.7	114.2 $\pm$ 6.7
*Crude fiber	70.8 $\pm$ 4.2	65.4 $\pm$ 3.8	61.2 $\pm$ 4.6	436.9 $\pm$ 12.7
*Ether extract	61.3 $\pm$ 1.6	54.4 $\pm$ 1.9	58.5 $\pm$ 1.4	105.4 $\pm$ 4.6
*Total ash	164.8 $\pm$ 7.2	168.6 $\pm$ 6.3	172.3 $\pm$ 6.6	42.1 $\pm$ 5.3
Concentrate intake (kg/cow/d)	<sup>a</sup> 2.3 $\pm$ .52	<sup>b</sup> 1.95 $\pm$ .61	<sup>c</sup> 1.57 $\pm$ .63	
Concentrate N intake (g/cow/d)	<sup>b</sup> 40.8 $\pm$ 6	<sup>a</sup> 55.8 $\pm$ 5.5	<sup>c</sup> 47.4 $\pm$ 4.6	
Grass dry matter intake (kg/cow/d)	<sup>b</sup> 7.84 $\pm$ .56	<sup>a</sup> 8.85 $\pm$ .45	<sup>b</sup> 8.27 $\pm$ .53	

Means with different superscripts in the same row show significant difference ( $P < 0.05$ ).

\*Values are as percentage of dry matter

**Table (3). Milk yield and fat yield of Sahiwal herd supplemented with urea molasses and fish meal in grazing condition.**

	<b>Control</b>	<b>Urea</b>	<b>Fishmeal urea</b>	<b><math>\pm</math>SEM</b>
<b>Milk yield</b> (kg/day/cow)	<sup>b</sup> 5.15	<sup>a</sup> 6.94	<sup>b</sup> 5.87	0.87
<b>Fat yield</b> (g/day/cow)	<sup>b</sup> 230.1	<sup>a</sup> 305.4	<sup>b</sup> 264.1	64.2
<b>Fat corrected milk yield</b> (kg/day/cow)	<sup>b</sup> 5.71	<sup>a</sup> 7.635	<sup>b</sup> 6.6	1.34

There was a significant increase in the milk yield and fat production in urea supplemented cows ( $P < 0.05$ ). Daily milk and fat yield presented here represents the average of 275 days observation in each cow. Means with different superscripts in the same row show significant difference ( $P < 0.05$ ).

There was a significant increase in milk yield and fat yield in urea supplement compared to urea with fish meal supplement and control. There was no significant difference between the control and urea with fish meal supplement. Actual milk yield, protein yield and 4% fat corrected milk yield were higher in urea supplement compared with urea with fish meal supplement or control. Neither the blood urea nitrogen nor the plasma protein content was affected by the treatments.

Protein content and fat content of milk were not affected by the treatments. Treatment effects on reproductive performance are presented in Table (4). Days to first standing heat was in the range of 50 days and there was no treatment effect. Days open and calving interval were lower in urea supplement compared to urea with fish meal supplement or control ( $P<0.05$ ).

There was no significant difference in number of services per conception in the current study. Numerous factors influence the number of services per conception, including time of detection of estrus and insemination. Nutrition seems to play a passive role on the success of service.

**Table (4). Reproductive parameters of Sahiwal herd supplemented with urea molasses and fish meal in grazing condition.**

	Control	Urea	Fish meal urea	±SEM
Number of cows	18	18	18	
Days to first standing heat	51.6	52.5	48.3	8.58
Services per conception	2.6	2.0	2.1	0.79
Days open	<sup>a</sup> 114.5	<sup>b</sup> 89.3	<sup>a</sup> 108.7	18.6
Calving interval	<sup>a</sup> 402.3	<sup>b</sup> 374.3	<sup>a</sup> 392.0	17.8

Means with different superscripts in the same row show significant difference ( $P<0.05$ ).

## Discussion

Nitrogen intake is a product of nitrogen content and amount of concentrate ingested. Urea supplement and urea with fish meal supplement group had urea as a source of nitrogen that increased the N content of the mixture. Concentrate intake was low in urea with fish meal supplement may be due to reduced palatability resulted from low level of copra meal. Copra meal contains residual oil that provides an appealing feed ingredient for cows. In spite of higher N content in urea with fish meal supplement low level of feed intake due to minimum level of copra meal resulted lower N intake. Increasing the crude protein content or inclusion of undegradable dietary protein (UDP) did not affect the fat content of milk in this experiment. Similar results have been reported in previous experiment (Klusmeyer, T.H., *et al.*, 1991).

Increase in N intake by ruminant increases the production of ammonia in the rumen that could be utilized for the growth and multiplication of rumen micro organism via enhanced protein synthesis (Mathur, O.P., *et al.*, 1994). This increased population will cause increased microbial activity in the rumen and hasten fiber digestion (Uthayathas, S., *et al.*, 1999) and possibly increase the grass intake. Increased influx of nutrients may be attributed for higher milk production in urea supplement. Several previous research findings also have indicated such improvement in milk production in response to feeding of non-protein nitrogen such as urea (Ghebrehwet, T., *et al.*, 1994). Production of milk did not affected by inclusion of UDP in this experiment, which agrees with recent work (Ellison, H.J., *et al.*, 1997; Charbonneau, E., *et al.*, 2007). Fish meal is highly resistant to ruminal degradation and expected to improve the total supply and amino acid profile and, thus, increase the milk production. Microbial protein supply may reach the maximum and supply of UDP is beneficial for milk protein synthesis in high producing dairy cows (Volden, J., 1999). Cows used in this experiment are of average producers and possibly dependent on the microbial protein supply alone. The strategy of enhanced microbial activity may be increased the utilization of grass to elevate the production.

Numerous studies indicate that the accuracy of early pregnancy diagnosis by milk progesterone is only about 80%. Reasons for this include: variation in estrus cycle length between cows, estrus detection errors, uterine disease, ovarian dysfunction, and early embryonic mortality, to name a few. In our experiment therefore, this assay was used to confirm non pregnancy rather than pregnancy. The advantage of this early confirmation of non-pregnancy prevents the further loss of early breeding opportunities. The probability of conception may be negatively associated with the magnitude and severity of negative energy balance in early lactation (Nebel, R.L. and McGilliard M.L., 1993). However, there are some work report poor correlation between nutrition and services per conception (Mondal, S.C., *et al.*, 2005). Calving interval of Sahiwal cows found to be longer than standards (Thorpe, W., *et al.*, 1994). Calving interval is a function of several factors mainly days open, number

of services per conception, length of postpartum anestrus and the heat detection accuracy and breeding efficiency (Kuhn, M.T., *et al.*, 2006). Lower days open than other groups in urea supplement indicates higher plane of nutrition. Acceptable level of all the reproductive parameters in this herd irrespective of treatment suggests the relative adequacies of the supplements providing nutrients to the body.

With ever increasing price of conventional feed ingredients supplementation with non conventional nitrogen such as urea become inevitable. Fertility parameters of a herd are one of the important factors determining the economic efficiency. The results of this study reveal that feeding urea to dairy cows may acquiesce beneficial on the reproductive performances. Thus the conventional concentrate mixture may be replaced by proposed urea based supplement in free grazing dairy without any adverse effect on the performance of cow. Further investigation is needed to draw a recommendation for partial substitution of copra meal with urea in molasses to be used in diets of cow.

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# **VETERINARY MEDICINE**



## **Concurrent Bovine Herpesvirus type1 (BHV-1) and Pasteurella Multocida Infections in Dairy Calves at Qassim Region, Central Saudi Arabia**

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**Abstract:** Bovine herpesvirus-1 infection (BHV-1) was diagnosed in calves, 4 - 15 weeks of age, in a dairy farm at Qassim region of central Saudi Arabia, during the spring of 2006. Fourteen calves out of a total of 70 cows and their calves died (i.e. 20% mortality rate). The clinical signs were severe respiratory distress, anorexia, fever and inflamed conjunctiva. On auscultation, wheeze and crepitation were heard on both sides of the chest. Affected calves had froth in the mouth with muco-purulent nasal and ocular discharges. Treatment with oxytetracyclin gave good response in few calves. Death usually occurred in 1-3 days after appearance of symptoms. Three of the calves that died of the disease were postmortemed. Necropsy findings were white spotted areas on the lungs that were also heavily congested and consolidated with frothy oedema on cut surface. Alveolar emphysema, bronchopneumonia and diffuse pleurisy were evident. The kidneys and liver were patchy pale in colour. Histopathological examination showed infiltration of neutrophils in the bronchioles and alveoli. Intranuclear inclusion bodies were found in bronchiolar and alveolar epithelium, in alveolar macrophages and in the liver cells. Coagulative necrosis was seen in the renal tubular epithelium. *Pasteurella multocida* cocco-bacilli, with its characteristic bipolar structure, were seen inside the alveoli. Diagnosis of BHV-1 and *Pasteurella* infections was confirmed by demonstration of viral antigens in the infected lung tissues by immunofluorescence and by microbiological methods respectively.

**Keywords:** BHV-1, *Pasteurella multocida*, pneumonia, calves, Qassim, KSA.

## Introduction

Herpesvirus belongs to the family herpesviridae that includes a large number of enveloped DNA viruses, many of which infect humans, cattle, sheep and goats (Quinn *et al.*, 1994; Muylkens *et al.*, 2007). Almost all herpes viruses express some common antigenic determinants and many produce Cowdry's type A intranuclear inclusion bodies and form multinucleated syncytial cells in infected tissues. Necrosis of cells induced by viral multiplication usually results in neutrophils and lymphocytes infiltration (Rubin *et al.*, 1999).

Diagnosis of herpesvirus infection is based on demonstration of characteristic gross and microscopic lesions induced by the virulent infection as well as by serological tests and virus isolation (Thompson, 1988). Bovine herpesvirus1 (BHV-1) infection is associated with several diseases in cattle such as infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), balanoposthitis, conjunctivitis, mastitis and encephalomyelitis. The virus subtypes are BHV-1-1 (respiratory subtype), BHV1-2 (genital type) and BHV1-3 (encephalitic type) (Quinn *et al.*, 1994).

Bovine herpesvirus1 infections are widely spread in feedlot cattle and the respiratory form is the most common (Muylkens *et al.*, 2007). The viral infection alone is not life threatening but it predisposes to secondary bacterial pneumonia which usually ends fatally (Jones *et al.* 1997). In this respect, *Mannheimia haemolytica* (formerly known as *Pasteurella haemolytica*) and *P. multocida* are the most common bacteria complicating herpesvirus infection in calves. They eventually cause fibrinous pneumonia and pleurisy, necrosis and sequestration of infected lung tissue and might obliterate the original pathology caused by herpes virus infection (Jericho and Langford 1978). Infection can be very severe in young calves resulting in a generalized septicemic disease. Also, pyrexia, ocular and nasal discharges, respiratory distress, diarrhoea, incoordination and eventually convulsions and death may occur in a short period of time following generalized viral infection (Anon, 2006).

In the present paper, the incidence of concurrent BHV-1 and *P. multocida* infection was confirmed in calves dying from acute pneumonia in a dairy farm at Qassim region of central Saudi Arabia. The gross pathology, histopathology and microbiological diagnosis of the disease were further described.

## Material and Methods

### Disease incidence

An acute febrile disease characterized by severe respiratory manifestations occurred in a dairy farm at Qassim region during the spring of 2006. The affected animals were mostly young calves aged 4 – 15 weeks. The total number of animals in the farm was about 70. The disease outbreak resulted in the death of fourteen calves (i.e. 20% mortality rate).

### Pathological methods

Detailed post-mortem examination was performed in three calves which died during the acute attack of the disease. Representative samples of organs showing lesions, particularly, the lungs, liver, kidneys and conjunctiva were immediately collected for histopathological studies. The tissues were fixed in 10% formol saline, processed in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin (H&E).

### Immunohistochemical methods

Herpesvirus1 antigen was demonstrated in lung and liver tissues by the use of fluorescence antibody technique (Silim and Elazhary, 1983). The tissues were dewaxed and brought into water. A volume of 20 µl of bovine herpesvirus-1 (infectious bovine rhinotracheitis) fluorescein-conjugated caprine polyclonal antiserum (VMRD –Inc. Pullman, WA, USA) was put onto the slides. One slide was left as treated control. After incubation for one hour at 37°C, the slides were washed in three changes of phosphate buffer saline (PBS) for 20 minutes and then dried at room temperature. Cover-slips were mounted onto the slides in 85% glycerin-PBS (pH 8.5). Examination of tissues was done with a fluorescence microscope (Olympus Optical Co., Japan).

### Bacteriological methods

Impression smears from infected lungs were stained with Gram and Leishman's stains for initial microscopic identification of the causative organisms. Tissue swabs were further obtained from infected lung tissues, eluted in 3 ml physiological saline and cultured onto casein-sucrose-yeast (CSY) blood agar plates

(Wijwardena *et al.*, 1986). After 24 hrs incubation at 37 °C, colonies were examined microscopically. *P. multocida* suspected colonies were tested for oxidase, catalase, indole and nitrate reduction and sugar fermentation tests. After species identification, the *Pasteurella multocida* isolates were tested for hyaluronidase production (Carter and Chengappa, 1980).

## Results

### Clinical picture

Infected animals showed clinical signs of severe respiratory distress, dyspnea, reluctance to move, anorexia, fever, cough and muco-purulent ocular and nasal discharges. The conjunctivae of both eyes were swollen and severely hyperaemic. The calves were treated for pneumonia with injectable oxytetracyclin and supportive intravenous (IV) fluid. The majority of calves recovered after treatment but some young calves (14 out of 70) continued to deteriorate and eventually died.

### Postmortem findings

Remarkable pneumonic alterations were observed in all of the three dead calves which were autopsied. Both the apical and diaphragmatic lobes of the right and left lungs were congested and consolidated with fibrinous inflammatory exudate. Some adhesions between the lungs and the pleura were also observed. When pressed, the cut surface of the lung was frothy and oozed blood and fibrin. The liver and kidneys were pale in colour and patchily congested.

### Histopathological and immunohistochemical findings

Histopathological examination showed that the lungs had bronchopneumonia with massive neutrophils infiltrations inside the bronchioles and alveoli. Some alveoli were filled with edema fluid. The epithelium of the bronchioles was sloughed into the lumen. Isolated necrotic lung tissues (sequestra) were seen in some sections. The alveolar epithelium and macrophages contained large intranuclear red inclusion bodies that had a halo around them. *Pasteurella* cocco-bacilli, with characteristic bipolar structure, were seen inside the alveoli (Fig. 1). Coagulative necrosis was seen in some hepatocytes and some liver cells contained large intranuclear eosinophilic inclusion bodies with a halo around them (Fig. 2a). The inclusion bodies were also found in the nuclei of epithelium of the conjunctiva where they occasionally occurred in pairs making the nuclear membrane looks highly dilated (Fig. 2b). The kidneys tubules showed coagulative necrosis but viral inclusions were not detected in them. By the use of fluorescence antibody technique, bovine herpesvirus antigen was demonstrated in the bronchiolar and alveolar epithelium as well as hepatocytes (Fig. 3a and 3b).

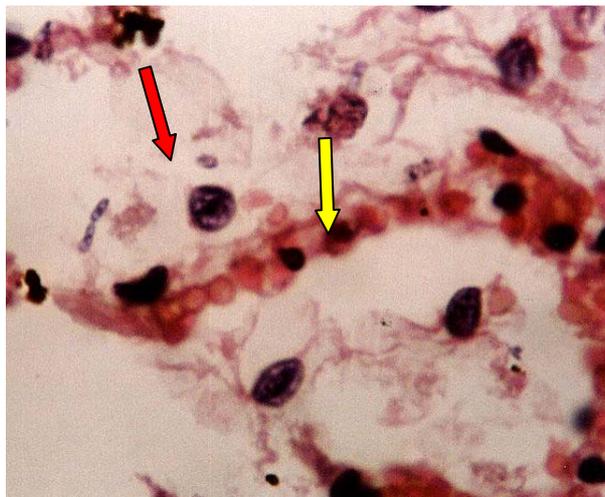
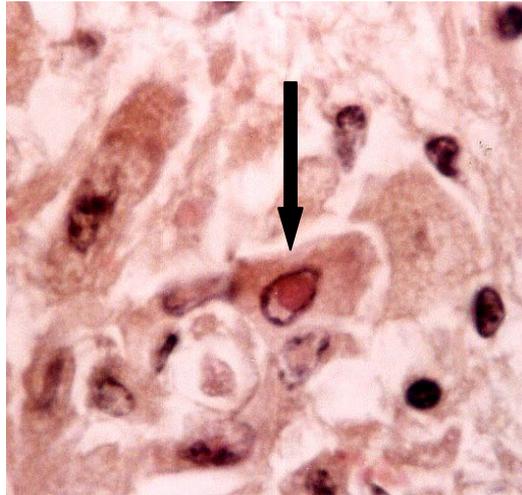
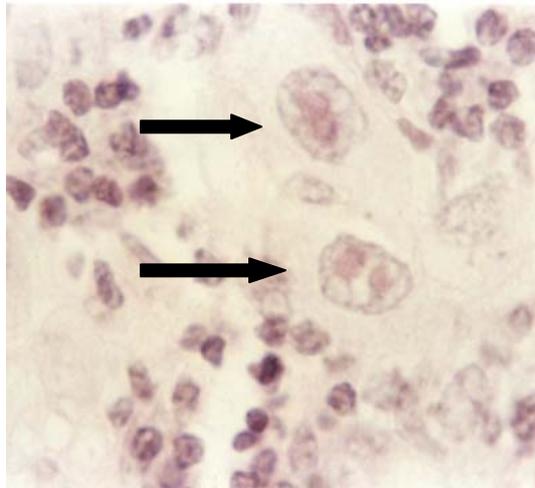


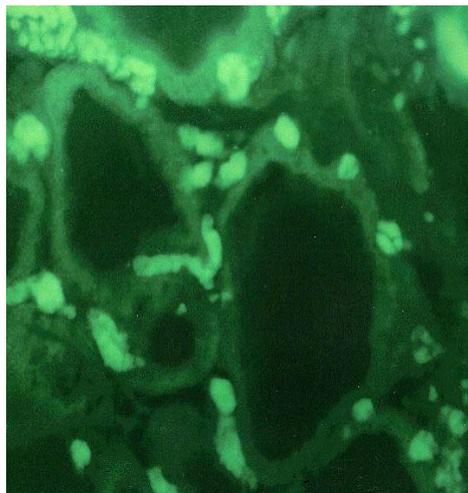
Fig. (1). Inclusion bodies (yellow arrow) and *Pasteurella* bacilli (red arrow) in the Alveoli (H&E x125).



**Fig. (2 a).** Intranuclear inclusion body in hepatocytes with a halo round it.



**Fig. (2 b).** Conjunctival epithelial cells having two intranuclear inclusion bodies with a large halo around them (H&E x400).



**Fig. (3 a).** Immunofluorescence staining of BHV antigen in alveolar epithelium.

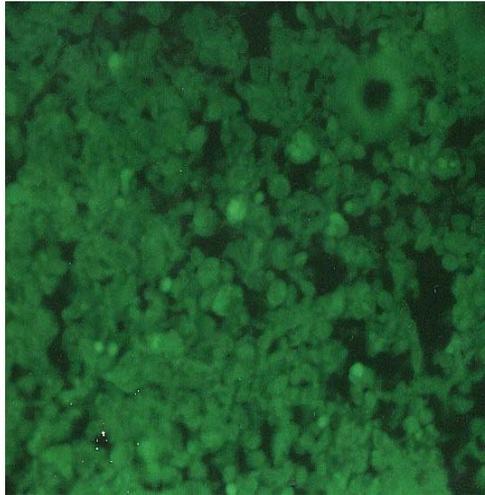


Fig. (3 b). Immunofluorescence staining BHV antigen in hepatocytes (X250).

### Bacteriological findings

Impression smears of infected lungs stained with Gram and Leishaman's stains showed Gram negative cocco-bacilli and bipolar staining coccoid forms, respectively. On CSY blood agar plates, *Pasteurella multocida* was identified as smooth and glistening colonies after 24 hrs incubation. Biochemically, the bacterial isolate was negative for hydrogen sulphide production, oxidase, indole, catalase and hyaluronidase production. It was positive for nitrate reduction. Glucose and sucrose were fermented with acid production only.

### Discussion

The role of viral bacterial interactions on the pathogenesis of bovine respiratory diseases has long been recognized (Carter, 1973). Respiratory viruses, such as BHV-1, usually damage the ciliated epithelium of the respiratory airways and predispose for entry of pathogenic bacteria. They also inhibit the mucociliary clearance making it possible for the invading bacteria to attach to the pulmonary epithelium (Kumar et al., 2002). The viruses infect the alveolar macrophages and the ciliated epithelium of the lungs thus disrupting its mucociliary transport and also inhibit the phagosome-lysosome fusion and inhibit the phagocytic ability of macrophages to kill bacteria (Jakab, 1982; Brown and Ananaba, 1988). A wide range of pathogenic organisms were involved but the common bacteria that complicate viral pneumonias mainly belong to the genus *Pasteurella* and *Mannheimia*.

In the present investigation, concurrent infection with BHV-1 and *Pasteurella multocida* was clearly confirmed in young dairy calves with a fatal respiratory disease. The gross and histopathological alterations seen in autopsied calves were mainly dominated by fibrinous-bronchopneumonia and pleurisy together with the presence of intranuclear inclusions in the epithelial cells and alveolar macrophages. These changes were highly indicative of BHV-1 infection complicated with pasteurellosis. In addition, the diagnosis was further confirmed by microbiological isolation of *Pasteurella multocida* and by demonstration of herpesvirus antigen in infected tissues of both lung and liver, using fluorescence antibody technique. It is worth mentioning that fibrinous bronchopneumonia and pleurisy were the most common lesions of pneumonic pasteurellosis in farm animals (Jones et al., 1997). The death of the calves in the involved farm was therefore attributed to primary herpesvirus infection complicated with secondary pasteurellosis.

It has been observed that the majority of reported cases of pneumonic pasteurellosis in calves were mostly due to *Mannheimia* (*Pasteurella haemolytica*) infection (Donachie, 2000; Mohamed and Abdelsalam, 2008). However, the bacteriological characterization in the present investigation only revealed the presence of *Pasteurella multocida*. The recovered isolates belonged to the B sero-type as they were hyaluronidase negative. The fact that they were not of the B2 or B6 sero-types would probably explain the occurrence of infection in young calves only. Besides, the disease was mainly manifested as a respiratory form and was not typical to that of haemorrhagic septicaemia (Kumar et al 1996). The results of the present investigation therefore provide

further confirmation of the catastrophic role of BHV-1 as a potentiator or initiator of secondary bacterial infections of the bovine lung.

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(قدم للنشر في ٢١/١٢/٢٠٠٨م؛ وقبل للنشر في ٢٢/٤/٢٠٠٩م)

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## Epidemiological Study on *Corynebacterium Pseudotuberculosis* In Imported and Native Sheep Ready For Slaughter During Hajj Season 1426H.

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**Abstract.** Caseous Lymphadenitis (CL) is a disease of sheep and goats that occurs worldwide with variable incidence. The causative agent is a bacterium, *Corynebacterium pseudotuberculosis* which caused caseous abscessation of external lymph nodes and in internal organs . A total of 1406 imported and 969 native sheep were examined clinically for the presence of external abscesses at different localities in Makkah El-Mokarrama during the Hajj season of 1426H. *Corynebacterium pseudotuberculosis* was widely prevalent in the native breeds ( 2.6% ) rather than in imported breeds ( 0.5% ). The most affected sites of sheep body were the superficial lymph nodes particularly the parotid 10 ( 31.25% ), prescapular 6 ( 18.75% ) and cervical 4 ( 12.5%). Out of 32 isolates, 16 pure cultures were identified and confirmed as *C. pseudotuberculosis*. Mixed infection were recognized in 4 cases. No temperature variations were recorded between the infected and control animals. Sensitivity test showed that Penicillin , Rifadin and Flumoxicillin were highly effective antibiotics to *C. pseudotuberculosis* , while Erythromycin, Doxycillin ( Vibramycin ) and Terramycin were moderately effective, whereas Ampicillin and Neomycin were slightly effective. Antibiotics of choice were applied after surgical interference for diseased sheep. Public health significance of the causative agent was discussed.

**Key words:** Caseous Lymphadenitis, sheep , *Corynebacterium pseudotuberculosis*, Hajj season, Antibiotics, sensitivity.

## Introduction

Caseous Lymphadenitis (CL) is a chronic bacterial infection that causes external and internal abscesses in sheep and goats. It is caused by *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*), which enters the body through a wound in the skin causing an infection and a slow growing, firm abscess. This infection may also travel to the regional lymph nodes causing a localized abscess there. *C. pseudotuberculosis* can survive for months in organic matter such as bedding. The typical pathogenesis of the disease is that the bacteria gain entry into the animal from a wound such as a shearing cut, or barbed a fence. The bacteria will then localize in an abscess that the animal walls off from the rest of its body. The clinical signs of the disease is one or more abscesses that are often located just beneath the skin. However, if the bacteria spreads throughout the bloodstream, abscesses may also develop in internal body organs such as the lungs resulting in thin ewes.

*C. pseudotuberculosis* (*C. ovis*) survives for up to 8 months in soil contaminated with pus (Augustine and Renshaw 1986). *C. pseudotuberculosis* was recovered from the external and internal, iliac, supramammary, deep inguinal, prefemoral and popliteal lymph nodes. The organism was also isolated from the cervical canal and abscesses embedded in the muscles of the thigh. On cut surface, nearly all the lymphoreticular tissue was found to be replaced by fibrous tissue surrounding the caseating green pus characterizing *C. pseudotuberculosis* infection (Fubini, *et al.*, 1983 and Pugh, 2002).

The incidence of *C. pseudotuberculosis* was 48% in prescapular, 30% in anterior cervical and 35% in prefemoral lymph nodes of 2-5 year – old sheep (Awad *et al.*, 1977), whereas, it was 53.22%, 26.2%, 14.4% and 6.04% in the prescapular, prefemoral, submaxillary and retropharyngeal lymph nodes in the examined 1545 sheep and goats, respectively (Ammar, 1983). In Kenya, Kuria and Ngatia (1990) detected Caseous lymphadenitis in 54 of 757 goats (7.13%) and 6 of 378 sheep (1.6%). The abscesses were commonest in the prescapular (68.25%) followed by the precrucial (14.28%) lymph nodes.

In Alberta (USA), Caseous Lymphadenitis (CLA) is one of the leading causes of lamb and mutton carcass condemnation. Serologic results confirmed a high (50-94%) incidence of exposure to *C. pseudotuberculosis*, in mature of unvaccinated sheep in southern Alberta (Stanford *et al.*, 1998). Shearing wounds and traumatized buccal mucosa are the major routes of infection. There is no variation in temperature between the diseased and control animals (Hassanien, 1998). *C. pseudotuberculosis* was isolated from two caseous lymphadenitis outbreaks in sheep and three Caseous lymphadenitis outbreaks in goats diagnosed in the Slovak republic. The isolated strains were tested for the biochemical activity and antibiotic susceptibility by Scepter kits and A.P.I. Zym, (Literak *et al.*, 1998). Mixed infection by other pyogenic organisms such as *streptococci*, *staphylococci*, *E. coli*, and *C. pyogenes* were isolated either separately or in association with *C. ovis* (Renshaw and Norman, 1979).

A total of 876 sheep from five flocks in north Jordan were selected to study the effect of shearing on the incidence of Caseous Lymphadenitis (CLA). The animals were divided into two age groups, sheep aged 1–2 years and those aged ≥3 years. Blood samples were collected from the animals at the time of shearing and again 6 months later. The prevalence of CLA was 6.59 % and 21.06 % in the 1–2-year and ≥3-year age groups, respectively. The overall prevalence among all ages was 15.3 %. In the shorn sheep, the incidence of CLA was 22.46 % and 9.47 % in the 1–2-year and ≥3-year age groups, respectively, and was significantly higher ( $P < 0.05$ ) in the 1–2-year age group. In the control animals, the incidence was 8 % and 5.26 % in the 1–2-year and ≥3-year age groups, respectively, and was significant ( $P < 0.01$ ) between the shorn (22.46 %) and control (8 %) animals of the 1–2-year age group (Al-Rawashdeh and Al-Qudah, 2000).

In Turkey, Burhan Çetinkaya *et al.* (2002) studied the prevalence of Caseous Lymphadenitis in sheep and goats slaughtered at the local abattoir in Elazig province, between September and December 2000. A total of 2046 sheep and 2262 goat carcasses were examined and 118 abscessed lymph nodes, 89 from sheep and 29 from goats, were collected. *Corynebacterium* spp. strains were isolated from 81.4% of the abscesses, giving an overall prevalence of 2.2%. The prevalence was 3.5% and 1.1% in sheep and goats, respectively. PCR on DNA extracted from 96 suspicious isolates, using a pair of *C. pseudotuberculosis* specific primers, was positive for 93 isolates. In addition, Kuria *et al.* (2001) reported that Caseous Lymphadenitis is a subacute disease with an incubation period of 8–9 days, but that might not be detectable serologically until after 15 days of infection.

The isolated strains of *C. pseudotuberculosis* were sensitive to Penicillin, Ampicillin, Erythromycin, Gentamycin, Tetracycline and Chloramphenicol (Ashfaque and compabell, 1981). They were also sensitive to Norfloxacin, Cephalothin, Methicillin, Kanamycin and Furazolidine and resistant to Ampicillin, Lincomycin and

Neomycin (Shippel *et al.*, 1993). Whereas, Hassanien (1998) stated that penicillin and Rifadin were highly effective antibiotics to *C. ovis* while, erythromycin, Doxycillin (Vebramycin) and Terramycin were moderate effective, Ampicillin and neomycine had slightly effective on *C. ovis* in vitro.

Human infection has been reported in few instances. The first reported case of *C. pseudotuberculosis* lymphadenitis from the United States was detected in a 30-year-old previously healthy man developed cervical adenopathy associated with mild constitutional symptoms. *C. pseudotuberculosis* was isolated in pure culture from lymph node tissue on two separate occasions, and small gram positive organisms were identified in both lymph nodes with tissue Gram stain. Of eight previously reported cases of *C. pseudotuberculosis* lymphadenitis in man, all but one had involved inhabitants of rural Australia, most of whom had contact with sheep. Necrotizing granulomas were usually observed. The patient described was an American urban dweller with a history of raw milk ingestion (Goldberger *et al.*, 1981). *C. pseudotuberculosis* lymphadenitis is a common disease in high country sheep in New Zealand and has previously been reported in humans overseas. The first human case is reported in New Zealand with a sheep confirmed as the infection source (House *et al.*, 1986). Another case of *C. pseudotuberculosis* lymphadenitis in a human being was reported in Spain by Bartolomé *et al.* (1995). A 34-years old previously healthy shepherd was attended for presenting a painful lymph node in right groin with one year of evolution. Cultures of an aspirate and ganglionar tissue yielded growth of *C. pseudotuberculosis* in pure culture. Moreover, In Australia, Mills *et al.* (1997) described suppurative lymphadenitis occurring in an adolescent boy who had contact with farm animals.

### **Material and Methods**

A total of 2375 imported and native sheep at Moesum area and various localities in Makkah El-Mokarram such as Al-Kakiah, Al-Sharraea, Bahrah and Al-Gommom were examined clinically for the prevalence of caseous lymphadenitis in sheep during the Hajj season of 1426H. Sheep animals showing external abscesses were isolated in a special enclosure to measure the temperature and collect the caseated material after surgical interference. The wool cover the affected area was sheared by a sterile curved scissors, and disinfected by 70% alcohol, then by 2% Tincture of iodine. By a sterile scalpel, the swollen lymph nodes was surgically opened, evacuated from the caseated material, then swabs were taken from the inside periphery of the lesion. After swabbing, the opened lymph nodes were surgically treated.

#### **Sampling**

Pus swabs were collected from enlarged superficial lymph nodes of 32 living sheep which apparently infected with caseous lymphadenitis. One swab was taken from each enlarged superficial lymph node after evacuation the contents under complete aseptic conditions. The collected swabs were directly transferred to the microbiological laboratory of the custodian of the two Holy mosques, Institute for Hajj research, department of environment and health researches, for different microbiological investigations.

#### **Laboratory examinations**

Direct smear films were prepared and stained by Gram's method, then examined under the microscope to detect Gram positive slender rods, arranged in Chinese letters (Mims *et al.*, 1993).

Isolation of the causative agent was done by spreading the pus swabs on 10 % sheep blood agar and nutrient agar plates as well as in nutrient broth tubes then incubated at 37°C for 48 hours. Suspected colonies were picked up and purified for further examinations (Wade Road *et al.*, 1982 and Elmer, *et al.*, 1997). Growth on blood agar at first was scanty, the colonies were small, flat, dry and grayish white in colour, folded and often showed concentric rings, while the growth on nutrient broth was granular, sometimes with a surface pellicle and showing the characteristic palisade arrangement. Suspected colonies were Gram positive coccoids or rods showing polymorphic shapes (Jensen, 1974). Identification and confirmation of the isolates were carried out by A.P.I tests for biochemical reactions (Hassanaien 1998).

#### **Antibiotics sensitivity test**

The effect of different antibiotics on *C. pseudotuberculosis* was studied by using sensitivity test to clarify the stronger and appropriate antibiotic used for treatment the diseased sheep. A total of 16 pure isolates were streaked on 10% sheep blood agar and incubated for 48 hours at 37 °C. Pure colonies were inoculated in

One ml of the nutrient broth and incubated at 37C° for two hours. 0.1ml of incubated broth was spread on the surface of nutrient agar plates and wait to dry for 15 to 30 minutes at 37C° .

Antibiotic discs consisting of 10 mg penicillin G, 15 mg Erythromycin, 10 mg Ampicillin, 30 mg Terramycin, 30 mg Neomycin, 30 mg Vebramycin (Doxycillin), 30 mg Rifadin (Rifampicin) and 30 mg flumoxcillin were left at room temperature for 30 minutes and carefully placed in the center of the incubated plates. The plates were incubated at 37C° for 24 hours. The zones of inhibition were measured and interpreted according to the method of Thornsberry and Baker (1981) and Monica Cheesbrough (2004).

## Results

The obtained results were recorded in the following tables:

**Table (1). The incidence of infected different sheep breeds with Caseous Lymphadenitis at various localities.**

Locality	Breeds	Number of examined sheep		Number of infected sheep	%	
Moesum	Imported	Rommany	630	1406	7	0.5
		Austorally	320			
		American	156			
		Swakni	300			
Al kakhiah	Native	Harry	440	969	25	2.6
Al - sharrae		Naeemy	170			
Bahrah		Nagdi	60			
Al - Gammom		Mixed Barki	149			
		Sawakni	150			
<b>Total</b>			2375		32	1.35

Table (1) showed that the incidence of caseous lymphadenitis in native breeds is increased about five times rather than the incidence in imported sheep. Whereas, Table (2) pointed out the distribution of infection within different lymph nodes in infected sheep either native or imported animals , where the parotid lymph node was the most affected lymph nodes in both infected imported and native breeds.

**Table (2). The prevalence of affected lymph nodes with Caseous Lymphadenitis in the examined sheep.**

Origin of animals	No. of examined sheep	Clinically infected Sheep	%	The affected lymph nodes								
				Parotid	Sub-lingual	Sub-mandibular	Cervical	Pre-scapular	Sub-scapular	iliac	Femoral	Poplital
Imported	1406	07	0.5	3	-	-	-	1	-	1	2	-
Native	0969	25	2.6	7	1	2	4	5	3	-	-	3
<b>Total</b>	2375	32		10	1	2	4	6	3	1	2	3
<b>Percentage</b>			1.35	31.25	3.1	6.25	12.5	18.75	9.4	3.1	6.25	9.4

## Discussion

Caseous Lymphadenitis (CL) is a chronic contagious disease affecting mainly sheep and goats This disease is also called pseudo-tuberculosis or often "abscesses". The incidence of the disease among sheep and goats in the Kingdom of Saudi Arabia increased considerably in the last few years. The main factors contributing to this increase among breeding and slaughter animals may be attributed to a large number of imported sheep particularly during Hajj season every year and the use of certain type of barely with sharp awns as animals feedstuff. The latter predispose the infection through abrasions in the mucous membrane of the alimentary tract.

The incidence of CL was 32 (1.35%) out of the examined 2375 imported and native sheep as recorded in Table (1). The obtained findings were in agreement with the results recorded by Awad *et al.* (1977), Kuria and Ngatia (1990) and Hassanien (1998). However, higher incidence of CL 15.3% and 3.5% were revealed in Jordan and Turkey by Al-Rawashdeh and Al-Qudah (2000) and Burhan Cetinkaya *et al.*, (2002), respectively. Concerning the prevalence of CL in the affected lymph nodes, it was found that the most affected lymph nodes of the body were parotid 10 (31.25%), followed by prescapular 6 (18.75%) then cervical 4 (12.5 %) followed by

subscapular and popliteal lymph nodes 2(6.25%), and sublingual and iliac lymph nodes 1(3.1%) for each as illustrated in Table (2). The most affected sites of CL in the examined sheep were parotid (31.8%) and prescapular lymph nodes (18.74%), which in accordance to the findings reported by Hassanien (1998). On the other hand, the prescapular lymph nodes were the predominant seats of infection, 48% (Awad *et al.*, 1977); 53.22% (Ammar, 1983) and 63.25% (Kuria and Ngatia, 1999).

CL is a worldwide chronic disease in goats and sheep; however, some animals within a herd appear to be very resistant to this disease. *C. pseudotuberculosis* causes CL. Goats and sheep can be infected by direct contact with this microorganism. The CL bacteria can be found in the soil of contaminated pens and pastures on feed and water troughs, and in shelters and other congregation points. The source of contamination is usually an abscess that has ruptured and drained onto various surfaces. Direct contact with a ruptured abscess by herd-mates will also spread the infectious bacteria from animal to animal. Animals can acquire infection orally when ingesting contaminated feed or grass. Upon infection, *C. pseudotuberculosis* will multiply and spread throughout the body via the bloodstream. Subsequently, lymph nodes and internal organs including the lungs, kidney and liver become infected and develop abscesses. The spinal cord can also develop CL abscesses. Once infected the animal is considered to be a carrier for life (Williamson, 2001, Pugh, 2002 and West *et al.*, 2002).

Identification of the isolated organisms was based on cultural, microscopically and biochemical parameters. However, *C. pseudotuberculosis* organisms were isolated and identified from 16 out of 32 samples collected from all infected animals showing enlargement of superficial lymph nodes, where, the organism was isolated from 3(42.86%), 4(57.14%), 3(60%), 2(50%) and 4(44.44%) out of 7, 7, 5, 4 and 9 infected sheep detected in Moesum, Al Kakia, Al-Sharraea, Bahrah and Al-Gammom areas as outlined in Table (3), respectively. Moreover, mixed infection accompanied with *C. pseudotuberculosis* such as *Staphylococcus* spp., *Streptococcus* spp., *Pasteurella* spp., and *Pseudomonas* spp. were detected. The present results were in acceptance to those reported by Renshaw and Norman (1979).

The temperature variations between infected and control animals either the imported or native sheep were recorded before incision abscesses in Table (4). The results showed that there was no variations in temperature between diseased and control animals, compared with normal temperature in sheep (38.9 – 40 C°) with an average of (39.5 C°) as described by Kelly (1974). Therefore, body temperature of infected sheep not useful as a scale for diagnosis of CL. Such observation was in agreement with the results obtained by Addo (1979) and Hassanien (1998). While, Gameel and Tartour (1974) pointed out that high temperature (41.1C°) was considered a symptom of diseased sheep during examination.

Moreover, in-vitro, antimicrobial sensitivity tests indicated that penicillin, rifadin and flomoxicillin were of highly effective, while, erythromycin, doxycillin and terramycin had moderately effect and ampicillin and neomycin had slightly effect on the isolated and identified strains of *C. pseudotuberculosis* collected from the infected sheep (Table, 5). Other study, showed that, *C. pseudotuberculosis* strains were sensitive to penicillin, ampicillin, erythromycin, gentamycin, tetracycline and chloramphenicol (Ashfaque and compabell, 1981), while they were resistant to ampicillin, lincomycin and neomycin and sensitive to norfloxacin, cephalothin, methicillin, kanamycin and furazolidine (Shipgel *et al.*, 1993).

*C. pseudotuberculosis* lymphadenitis in human beings is a rare entity that principally affects persons in contact with animals, principally sheep. Few instances of human infection has been reported in United States of America (Goldberger *et al.*, 1981); in New Zealand (House *et al.*, 1986); in Spain Bartolomé *et al.* (1995) and in Australia (Mills *et al.*, 1997). So, *C. pseudotuberculosis* infection in man should be considered in the differential diagnosis of localized granulomatous lymphadenitis.

#### **Particular recommendations during Hajj seasons**

Quarantine measures should be applied for all imported sheep. As well as, ante mortem examination should be done carefully for every animal to detect the infected sheep. If the infection is severe and involve more than two superficial lymph nodes, and accompanied by emaciation, the animal should be rejected and considered unfit for human consumption as well as unsuitable for Hadie and Adahi. Any detected cases should be isolated to prevent spreading of infection between thousands of sheep animals during Hajj season. Treated cases with antibiotics should be known and put in consideration the withdrawal periods for each applied antibiotic to avoid hypersensitivity and bacterial resistance.

**Table (3). Occurrence of *C. pseudotuberculosis* in the examined samples collected from sheep in different localities.**

Localities	Samples	Pus from caseated lymph nodes		
		No.	+ve	%
Moesum		7	3	42.86
Al kakiah		7	4	57.14
Al - sharraea		5	3	60.00
Bahrah		4	2	50.00
Al -Gammom		9	4	44.44
Total		32	16	50.00

**Table (4). Temperature variation between the infected and control animals.**

Breeds		Range C°	Average
Important animal	Romamany	39.9 - 40.4	40.15
	Austorally	39.3 - 41.1	40.20
	American	39.1 - 40.1	39.60
	Sawakni	39.3 - 41.3	40.30
Native animals	Harry	39.3 - 40.6	39.9
	Naeemy	39.9 - 40.8	40.35
	Nagdi	38.2 - 41.2	39.70
	Mixed barki	38.7 - 40.5	39.60
	Sawakni	39.7 - 40.1	39.90
<b>Control</b>		<b>39.3 - 40.2</b>	<b>39.80</b>

NB : Normal temperature in sheep = 38.9 – 40 C° with an average ( 39.5 C° ) Kelly (1974).

**Table (5). Antibiotic sensitivity test for *C. pseudotuberculosis*.**

Test	Number of isolates	Different Antibiotic							
		Penicillin	Rifadin (rifampicin)	Flumoxicillin	Erythromycin	Doxycillin	Terramycin	Ampicillin	Neomy-cin
Sensitivity	16	++++ S	++++ S	++++ S	+++ I	+++ I	+++ I	++ R	++ R

N.B The test applied on the 16 pure isolates of *C. pseudotuberculosis*, + + + + : Highly sensitive to antibiotic , S = Sensitive to antibiotic, + + + : Moderately sensitive to antibiotic, I = Intermediate sensitive to antibiotic, + + : Slightly sensitive to antibiotic, R = Resistant to antibiotic, According to Moica cheesbrough ( 2004 )

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## **Evaluation of the GnRH-PGF<sub>2</sub>α-GnRH synchronizing program in cyclic and non-cyclic Rahmani ewes**

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**Abstract.** The aim of the present study was to evaluate the efficiency of the GnRH-PGF<sub>2</sub>α-GnRH program to synchronize the time of estrus and ovulation in cyclic (CYC) and non-cyclic (NCY) ewes. A total number of 63 ewes were used in two experiments. In the first experiment, 7 CYC and 10 non-cyclic NCY ewes were treated with GnRH-PGF<sub>2</sub>α-GnRH protocol. The ovarian structures were ultrasonographically examined and blood samples were obtained for determination of serum progesterone (P<sub>4</sub>) level. In the second experiment, 46 ewes were examined for ovarian cyclicity and allocated into group A (cyclic treated, CYC-T, n=16), group B (cyclic non-treated, CYC-NT, n=10), group C (non-cyclic treated, NCY-T, n=11), and group D (non-cyclic non-treated, NCY-NT, n=9). Ewes of groups A and C were treated with the same protocol as in experiment 1. Ewes of all groups were joined on the 5<sup>th</sup> day with fertile rams. The results showed that, low proportion (28.6%) of CYC and none (0.0%) of NCY ewes ovulated in response to the 1<sup>st</sup> GnRH. By day of luteolysis, 57.1% of CYC ewes were presented with a mature CL and 85.7% of them had a large follicle. None of the NCY ewes had a CL, and 60% had a large follicle. All CL regressed efficiently in response to PGF<sub>2</sub>α administration. The percentages of ewes that ovulated in response to the 2<sup>nd</sup> GnRH were 71.4% and 20% for CYC and NCY groups (P=0.02), respectively. The incidences of estrus expression were 62.5%, 40%, 9.1%, and 0.0% for CYC-T, CYC-NT, NCY-T, and NCY-NT, respectively (P=0.001). The pregnancy rates were 31.3%, 20%, 0.0%, and 0.0% for the same groups, respectively (P=0.02). In conclusion, the efficiency of the GnRH-PGF<sub>2</sub>α-GnRH protocol for estrous synchronization depended mainly on the ovarian cyclicity.

**Keywords:** Ewes, GnRH, Prostaglandin, Estrous synchronization, Ovulation

## Introduction

A treatment protocol consisted of an initial administration of a GnRH followed 5 days later by PGF<sub>2</sub>α, and 36 hrs later by a second GnRH injection was developed to synchronize ovulation in cyclic ewes (Deligiannis *et al.*, 2005). About half of the treated ewes conceived and maintained pregnancy when inseminated. Whether this protocol would function in non-cyclic ewes, has not yet been investigated. Ovarian follicular dynamics and luteal development occurring during this synchronizing protocol in ewes have not been previously recorded.

The protocol has been originated in cattle by Pursley *et al.* (1995), where the 1<sup>st</sup> GnRH was intended to ovulate large functional follicle and to induce a new follicular wave, as well as to increase the percentage of animals synchronized to a single injection of PGF<sub>2</sub>α. The PGF<sub>2</sub>α was injected to regress all CL. A second GnRH dose was injected to ovulate the preovulatory follicle at a precise time. A similar protocol, using the same time intervals as in cattle, has been recently practiced in goats during the breeding season, resulting in a 58% kidding rate (Holtz *et al.*, 2008).

The recorded effects of exogenous GnRH on the ovarian follicular dynamics and luteal outcomes in sheep were considerably controversial. Season (Bartlewski *et al.*, 2001, Liu *et al.*, 2007), days of estrous cycle (Whitmore *et al.*, 1996, Rubianes *et al.*, 1997), days postpartum (Mitchell *et al.*, 2003), nutritional condition (Mitchell *et al.*, 2003), and method of administration (Ainsworth *et al.*, 1982, McLeod *et al.*, 1982) were influencing factors for ovarian follicular responsiveness to exogenous gonadotrophins. The aim of this study was to characterize ovarian dynamics in cyclic and non-cyclic Rahmani ewes during different steps of GnRH-PGF<sub>2</sub>α-GnRH protocol and to estimate the reproductive performance of ewes treated with this program.

## Material and Methods

### Animals and management

A total of 63 multiparous fat-tailed Rahmani ewes aged 2-5 years-old and weighing 36-41 kg were used in this study. All ewes lambed in February and nursed their lambs for 8 weeks. All delivered spontaneously without complications. The animals were housed in a semi-opened yard in the animal farm of Al-Azhar University, Assiut Campus, and fed a concentrate diet.

### 2.2. Experiment 1

After weaning by 2-3 weeks (May/June, 32.72±0.7°C average atmospheric temperature and 45.32±1.0 % average relative humidity), the ovaries of 17 ewes were ultrasonographically examined (real-time, B-mode, diagnostic scanner equipped with a transrectal 5/7.5 MHz linear array transducer, Hitachi, EUB-405B, Tokyo, Japan) twice at the 10<sup>th</sup> day apart to assess the ovarian cyclicity, based on the presence of a mature corpus luteum (CL) in one of either examinations that confirmed later by serum progesterone (P<sub>4</sub>) analysis. Accordingly, the ewes were allocated into two groups, group 1 (cyclic, CYC, n=7) and group 2 (non-cyclic, NCY, n=10). All ewes were intramuscularly injected with 8 µg GnRH (Buserelin, Receptal, Intervet International B.V., Boxmeer, Holland) on day 0 (beginning of the program), 15 mg PGF<sub>2</sub>α (Dinoprost, Lutalyse, Pharmacia, Belgium) on day 5, and 8 µg GnRH after 36 hrs later (Deligiannis *et al.*, 2005). Ultrasound examinations were performed once daily from day 0 to day 5, and at 12 hrs intervals thereafter until the expected time of ovulation (or for a maximum of 72 hrs). All visible follicles and CL were measured, and mapped individually for each ewe. Ovulation was considered to have occurred when a large growing antral follicle that had been identified and followed for several days was no longer observed. Emergence of a follicular wave was defined as occurring on the day on which the retrospectively identified dominant follicle was 2 mm. The CL was examined and an image of the largest cross-sectional area was estimated. Luteal regression following PGF<sub>2</sub>α administration was considered when serum P<sub>4</sub> level was <1 ng ml<sup>-1</sup>. The mean number of small (2-2.9 mm in diameter), medium-sized (3-5 mm in diameter) and large follicles (> 5 mm in diameter), were recorded. The following ovarian characteristics were determined and compared between groups, (1) ovulation rates after the 1<sup>st</sup> and 2<sup>nd</sup> GnRH injected dose; (2) diameter of the ovulatory follicles; (3) interval from treatment to emergence of a new follicular wave after the 1<sup>st</sup> GnRH injected dose ; (4) number and diameter of the CL; (5) luteal regression rate after PGF<sub>2</sub>α injection; and (6) follicular population. Blood samples were obtained via jugular venipuncture on d 0, 5, 7, 12 and 18 of treatment, the serum was harvested and stored at -20 for serum P<sub>4</sub> analysis.

## Experiment 2

To evaluate the relative importance of the ovarian cyclicity and treatment, as well as their interaction on reproductive performance of ewes, a 2 x 2 factorial experiment (cyclicity x treatment) was designed utilizing the rest of ewes of the same flock (n=46). The ewes were examined for ovarian cyclicity as in experiment 1 and allocated randomly into group A (cyclic treated, CYC-T, n=16), group B (cyclic non-treated, CYC-NT, n=10), group C (non-cyclic treated, NCY-T, n=11), and group D (non-cyclic non-treated, NCY-NT, n=9). Each ewe of groups A and C were treated with the same protocol as experiment 1. All ewes were joined on the 5<sup>th</sup> day of the program with fertile rams for 3 days for mating. The proportion of ewes showing estrus behavior was recorded. Pregnancy diagnosis was performed 45 day after mating using the ultrasound technique and the pregnancy rate of the induced / synchronized estrus were calculated and compared.

### P<sub>4</sub> estimation

The concentration of progesterone (P<sub>4</sub>) in serum was determined by RIA utilizing kits provided by Diagnostic System Laboratory Co. (DSL, Catalogue No. 3900, Texas, USA). The sensitivity of the assay was 0.12 ng P<sub>4</sub>, and the coefficient of variance of intra- and interassay were 4.8 and 9.2%, respectively.

### Statistical analysis

The data were presented in mean  $\pm$  S.E.M, and statistical analysis was carried out using SPSS program, version 11.0 (2001). Differences in ovulation rates after GnRH treatment, luteal regression rates after PGF $2\alpha$  treatment between CYC and NCY ewes, estrus expression and pregnancy rates were evaluated by Chi-square test. Repeated data including follicular population were analyzed by ANOVA for repeated measurements. T-test was used to compare means for follicle and CL diameters within the examination dates, and the interval from treatment to ovulation and to wave emergence. Level of significance was set at P<0.05.

## Results

### Ovarian response to the 1<sup>st</sup> GnRH

Ovarian response of CYC and NCY ewes to GnRH-PG-GnRH program is summarized in (Table 1). Low proportion of CYC (28.6%) and none of NCY ewes (0.0%) ovulated in response to the 1<sup>st</sup> GnRH. Recruitment of new follicular waves was observed only in CYC ewes (71.4%), P=0.007. In CYC ewes, four CL (2 newly formed and 2 old) decreased gradually in size between day 0 and day 5 of treatment.

**Table (1). Ovarian response of cyclic (CYC) and non-cyclic (NCY) ewes to GnRH- PGF $2\alpha$  -GnRH protocol.**

	CYC n=7	NCY n=10
<b>Ovarian finding on D 0:</b>		
CL n	5/7a	0/10 <sup>b</sup>
CL diameter mm	11.3 $\pm$ 0.3 (10.5-12.5)	-
F >5 mm n	5/7a	2/10a
Diameter of F >5mm	5.6 $\pm$ 0.2 (5.0-6.3)	5.1 $\pm$ 0.1
<b>Response to 1<sup>st</sup> GnRH</b>		
Ovulation n	2/7a	0/10a
Time to ovulation h	48 $\pm$ 6.5 (36-60)	-
Ovulatory F diameter mm	6.0 $\pm$ 0.1 (5.9-6)	-
Follicular waves n	5/7a	-
Time to follicular wave h	28.8 $\pm$ 4.8 (24-48)	-
<b>Ovarian finding on D 5</b>		
CL n	3/7a	0/10 <sup>b</sup>
Diameter of CL mm	13.9 $\pm$ 0.4 (13-15.6)	-
F >5 mm n	6/7a	6/10a
Diameter of F >5 mm	6.0 $\pm$ 0.4 (5.5-7.7)	5.6 $\pm$ 0.5 (5.1-6.5)
<b>Response to PG</b>		
Regression of CL mm d <sup>-1</sup>	3.5 $\pm$ 1.0 (1.9-6.6)	2.1 $\pm$ 1.0
Growth of LF mm d <sup>-1</sup>	0.9 $\pm$ 0.3a (0.0-2.4)	0.2 $\pm$ 0.1a (0.0-0.4)
<b>Response to 2<sup>nd</sup> GnRH</b>		
Ovulation n	5/7a	2/10 <sup>b</sup>
Diameter of ovulatory F	6.6 $\pm$ 0.2 (6.1-7.7)	6.2 $\pm$ 0.1 (6.1-6.3)
Time to ovulation h	18 $\pm$ 2.6 (12-24)	24 $\pm$ 0.0
Growth rate of ovulatory F mm d <sup>-1</sup>	0.4 $\pm$ 0.1 (0.0-0.1)	0.75 $\pm$ 0.0

CL: corpus luteum; F: Follicle; Data in mean $\pm$ S.E.M. Values with different letters (a,b) differ sufficiently (P< 0.05).

### Ovarian structures on the 5<sup>th</sup> day:

By day of luteolysis, 57.1% of CYC ewes were presented with a mature CL ( $P_4 > 2.0 \text{ ng mL}^{-1}$ ), and 85.7% of them had a large follicle ( $> 5 \text{ mm}$  in diameter). None of the NCY ewes had a CL, and 60% had a large follicle..

### Ovarian response to $\text{PGF}_2\alpha$

All presented CL were regressed efficiently ( $P_4 < 1 \text{ ng mL}^{-1}$ ) in response to  $\text{PGF}_2\alpha$  injection on the 5<sup>th</sup> day.

### Ovarian response to the 2<sup>nd</sup> GnRH

Proportions of ewes that ovulated in response to the 2<sup>nd</sup> GnRH were 71.4% and 20% for CYC and NCY groups, respectively ( $P=0.02$ ). In CYC ewes, ovulation occurred over a period of 12 hrs, starting 12 hrs after the 2<sup>nd</sup> GnRH.

### Follicular population

Day of treatment had no significant influence on the number of small, medium or large sized follicles in CYC and NCY groups (Fig 1). The number of small follicles was significantly greater in CYC than in NCY ewes on D 0 ( $P=0.001$ ) and D 6 ( $P=0.04$ ) of treatment.

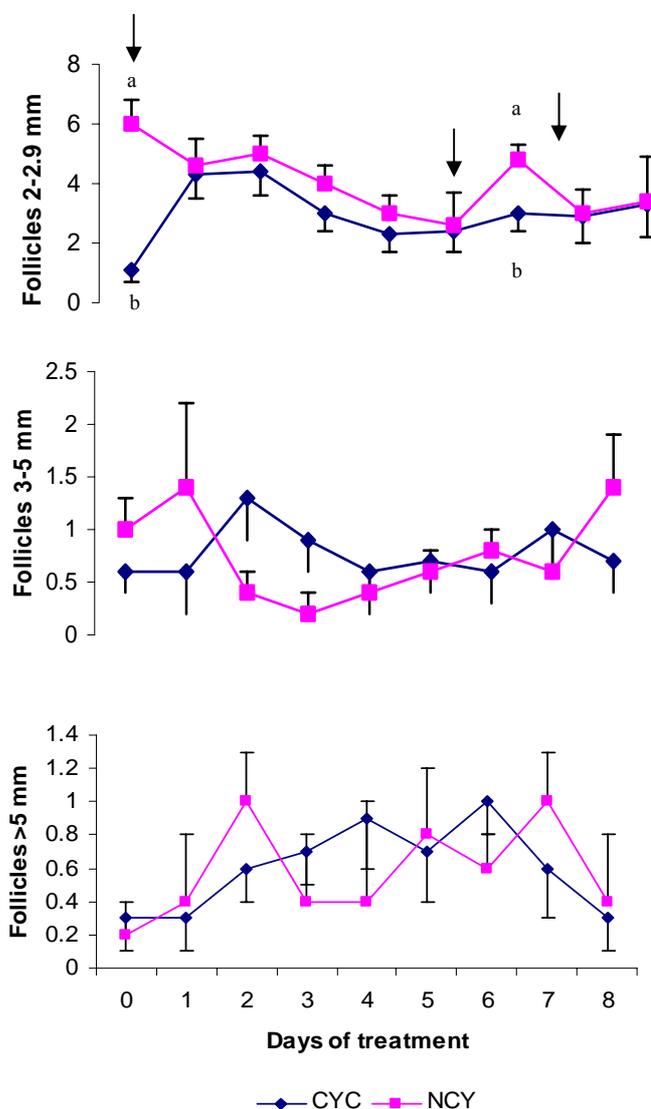


Fig. (1). Fluctuation in the mean number ( $\pm$ S.E.M.) of small (2-2.9 mm), medium (3-5 mm), and large sized follicles ( $> 5 \text{ mm}$ ) in response to GnRH- $\text{PGF}_2\alpha$ -GnRH treatment in cyclic (CYC,  $n=7$ ), and non-cyclic (NCY,  $n=10$ ) ewes. a,b: values differ significantly ( $P < 0.05$ ).

### Progesterone concentration

The mean serum P<sub>4</sub> level in CYC and NCY ewes treated with the program is shown in Fig 2. After the first GnRH treatment in CYC ewes, the mean serum P<sub>4</sub> level increased from day 0 up to day 3, then it decreased gradually until day 5 (P=0.04). By this day, only 3 out of the 7 CYC ewes had a P<sub>4</sub> values > 2.0 ng mL<sup>-1</sup>. After PGF<sub>2</sub>α injection on day 5, the mean serum P<sub>4</sub> level decreased sharply (P=0.001). In NCY ewes, the mean serum P<sub>4</sub> level remained basal during treatment.

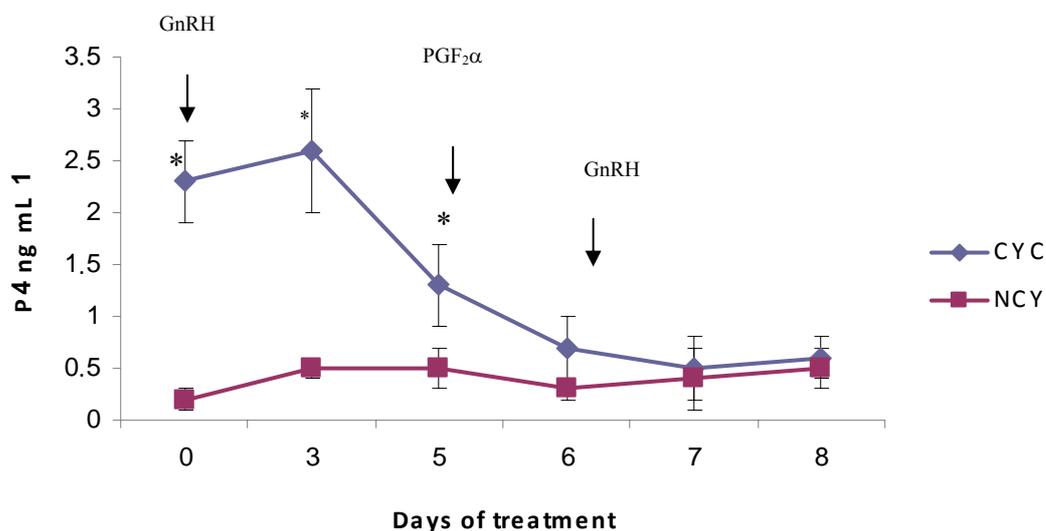


Fig. (2). Serum progesterone concentrations (P<sub>4</sub>, mean ± SEM) in cyclic (CYC, n=7) and non-cyclic (NCY, n=10) ewes treated with GnRH-PGF<sub>2</sub>α-GnRH program. \*Values differ significantly (P< 0.05).

### Reproductive performance

The effect of ovarian cyclicity x treatment on reproductive performances is shown table 1. Estrus expression and pregnancy rate were significantly greater in CYC-T, and CYC-NT than in NCY-T and NCY-NT, (P < 0.05). No significant differences were observed between CYC-T and CYC-NT. There was no significant interaction between ovarian cyclicity and treatment on estrus expression and pregnancy rate. The mean interval from PGF<sub>2</sub>α injection to the onset of estrus and the mean duration of estrus in CYC-T ewes were 57.6±9.9 h and 33.6±9.8 h, respectively.

### Discussion

The present study described the efficiency of a novel (GnRH-PGF<sub>2</sub>α-GnRH) program in inducing / synchronizing the time of ovulation in cyclic and non-cyclic ewes. The results obtained showed that this program was not functioning in the NCY ewes, supporting the results of Ainsworth *et al.* (1982) who ruled out the use of GnRH as a single injection for induction of cyclic ovarian activity. Bartlewski *et al.* (2001) and Liu *et al.* (2007) reported that when ovulations were induced with GnRH during anestrus, a proportion of ewes produced fewer CL than ovulated follicles or developed short-lived CL. In addition, many of ovulatory-sized follicles did not ovulate in response to GnRH, and some of these follicles continued to grow and form cystic-like, luteinized follicles. These observations were partially supported in this study. Others have suggested that ovulation with subsequent normal luteal function occurred in seasonally anoestrous ewes treated with small-dose multiple injections of GnRH (McLeod *et al.* 1982). Unfortunately, failure of NCY ewes to respond to first GnRH was a major flaw in this program. To make this program effective, NCY ewes might be sensitized for the first GnRH using exogenous progesterone. It has been reported that progesterone priming apparently sensitized GnRH treated seasonally anestrous ewes and increased their response in estrus and pregnancy rates (Southee *et al.*, 1988, Husein and Kridli, 2003, Bramley *et al.*, 2005).

Even in the CYC ewes, the required high progesterone levels at time of prostaglandin administration were relatively low. Such proportion of successfully synchronized females was definitely lower than that previously published for cattle and goats using similar programs (Pursley *et al.*, 1995, Holtz *et al.*, 2008). Season when this experiment was conducted (May/June), days postpartum (about 2.5 months), breed used, and day of estrous cycle (undefined) might be factors that influenced the ovarian response of CYC ewes to the exogenous GnRH treatment. In this concern, it has been reported that treatment of ewes with GnRH on day 10 of the estrous cycle resulted in formation of corpora haemorrhagica in the majority of ewes within 2 days after injection of GnRH (Keisler and Keisler, 1989), while treatment during metestrus failed to alter systemic concentrations of oxytocin, PGF<sub>2</sub> $\alpha$ , and progesterone (Whitmore *et al.*, 1996). Furthermore, whereas follicles presented during the early luteal phase were capable to ovulate and form fully functional CL in response to exogenous GnRH, follicles presented during the late luteal phase failed to ovulate in response to the GnRH (Rubianes *et al.*, 1997). We suggest that, CYC ewes may be pre-synchronized using double PGF<sub>2</sub> $\alpha$  injection 9-11 days apart prior to the first GnRH treatment, which should be administered during early or mid-luteal phase.

Differences in follicular populations between CYC and NCY ewes observed in this study seemed to be related to the ability of the first GnRH to recruit new follicular waves in the CYC group. The incidences of estrus expression, ovulation and pregnancy of the present program are relatively low, supporting the outcome of the first experiment and indicating that this program should be modulated before being practiced in the sheep industry. Dose of the drugs, time intervals between treatments, ovarian status at administration, body condition of ewes, and lambing interval are factors that should be considered in protocol application.

In conclusion, the efficiency of the GnRH-PGF<sub>2</sub> $\alpha$ -GnRH for synchronizing estrus and ovulation depended on the ovarian cyclicity. Synchronization is not effective in NCY ewes and should be improved before use in CYC ewes.

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