CENTRO REGIONAL DE INVESTIGACION CARILLANCA
TEMUCO - IX REGION DE LA ARAUCANIA

GOBIERNO DE CHILE
MINISTERIO DE AGRICULTURA
INSTITUTO DE INVESTIGACIONES AGROPECUARIAS

FUNDACION PARA LA INNOVACION AGRARIA - FIA

INFORME TÉCNICO Y DE DIFUSIÓN

CÓDIGO FIA BID-CD-V-2005-1-P-032

"4TH INTERNATIONAL SYMPOSIUM ON THE MOLECULAR BREEDING OF FORAGE AND TURF"

ABERYSTWYTH - REINO UNIDO

3 AL 7 DE JULIO DE 2005

INIA – CARILLANCA

NOVIEMBRE DE 2005
TEMUCO-CHILE

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INFORME TÉCNICO

Fecha de entrega del Informe
28 de Noviembre de 2005
Nombre del coordinador de la ejecución
Fernando Mauricio Ortega Klose
Firma del Coordinador de la Ejecución

1. ANTECEDENTES GENERALES DE LA PROPUESTA

Nombre de la propuesta

4th International Symposium on the Molecular Breeding of Forage and Turf, 3 a 7 de julio 2005.

Código

BID-CD-V-2005-1-P-032

Entidad responsable

Instituto de Investigaciones Agropecuarias(INIA)

Coordinador(a)

Fernando Ortega Klose

Tipo de iniciativa(s)

	Gira		Beca	X	Evento		Consultores		Documentos

Fecha de realización (inicio y término)

Inicio : 1 de Julio de 2005

Término : 9 de julio de 2005

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RESUMEN DE LA PROPUESTA

Como investigador de INIA Carillanca me he especializado en Fitomejoramiento de Forrajeras, línea sobre la cual he desarrollado proyectos de Mejoramiento Convencional y de Aplicaciones Biotecnológicas en distintas especies. La propuesta realizada obedece a la necesidad e interés de actualizar y fortalecer mis conocimientos en las temáticas que se plantearon en el Simposio, a objeto de reforzar el desarrollo científico y tecnológico de esta área que ha sido poco desarrollada en Chile.

De acuerdo a lo planificado, la actividad permitió conocer los avances en mejoramiento genético molecular de especies forrajeras (MMSPF). **En el simposio** se comenzó con presentaciones que abordaron los objetivos actuales y beneficios del MMSPF para posteriormente en las otras sesiones conocer el avance en el desarrollo de herramientas para alcanzar tales objetivos.

En cuanto a los objetivos, estos son cada vez más específicos en relación a los caracteres a mejorar pero a su vez, más integrales en cuanto a su relación con el animal, ambiente y salud humana. El mapeo, la selección asistida por marcadores y la transgenia permiten abordar objetivos cada vez más específicos y piramidar genes de interés. Por otro lado, el mejoramiento genético ya no termina con la evaluación de la producción de forraje, semilla o calidad del cultivar, sino que tiene un enfoque integral ya que apunta a crear cultivares y evaluar sus atributos específicos que afectan el consumo, la productividad animal y la calidad composicional de los productos animales, la forma como estos productos animales satisfacen la demanda humana y afectan su salud, velando a su vez por la disminución de los impactos negativos de la producción ganadera en el ambiente.

Una vez finalizado el simposio se visitó el centro de investigación de IGER, oportunidad en que se atendió un taller de trabajo que dio lugar a una red internacional de trabajo en tréboles (International Trifolium Network, ITN). Por otro lado, se sostuvo reuniones con investigadores del departamento de Producción Animal y microbiología para conocer el grado de avance en líneas de trabajo de interés común.

La participación en el simposio permitió fortalecer contactos ya existentes y establecer nuevos contactos con los grandes centros de investigación que lideran la temática. El grado en que en Chile podamos incorporarnos a estas líneas de trabajo dependerá de la postulación y aprobación de proyectos.

Al regreso a Chile, los nuevos conocimientos captados fueron difundidos en tres charlas a un total de 75 productores y profesionales. También se difundieron por medio de artículos en revista Campo Sureño del diario Austral y se despachó para publicación en revista Nuestra Tierra y Chile Agrícola.

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2. ALCANCES Y LOGROS DE LA PROPUESTA GLOBAL

Problema a resolver, justificación y objetivos planteado inicialmente en la propuesta

Objetivo General

Conocer las nuevas tendencias de los principales grupos de investigación mundial en el desarrollo y uso de herramientas biotecnológicas en el mejoramiento genético de especies forrajeras y contribuir con información actualizada al país respecto a estas materias.

Objetivos Específicos

- Conocer los nuevos enfoques y objetivos en mejoramiento genético de especies forrajeras y ver la forma como se están incorporando las aplicaciones biotecnológicas en estos nuevos lineamientos.
- Actualizar conocimientos respecto a los avances en especies forrajeras en desarrollos biotecnológicos tales como mapeo, análisis QTL, análisis funcional de genes, selección asistida por marcadores, y transformación genética.
- Intercambiar experiencias respecto a los puntos anteriores, estudiar factibilidad de aplicación en Chile y establecer contactos con posibles contrapartes de grupos de investigación internacionales para la presentación y formulación de proyectos de I&D.
- Difundir la información entre los investigadores, productores y la industria productora y distribuidora de semilla forrajera y de prados nacional.

Objetivos alcanzados tras la realización de la propuesta

Se alcanzó el objetivo general y los objetivos específicos originalmente planteados. Adicionalmente nos incorporamos a una red internacional de trabajo en tréboles (International Trifolium Network, ITN).

Resultados e impactos esperados inicialmente en la propuesta

Resultados:

- *Corto Plazo:* **Haber transferido los conocimientos adquiridos en forma directa a investigadores, productores y la industria nacional productora y distribuidora de semilla forrajera y de prados.**

- *Mediano Plazo:* Haber consolidado contactos para el establecimiento de alianzas y redes de apoyo en las líneas de investigación de biotecnología, recursos genéticos y fitomejoramiento de especies forrajeras.

- *Largo Plazo:* Haber concretado la ejecución de proyectos I&D en conjunto con las alianzas establecidas.

Impactos:

- Incorporación de nuevas herramientas y fortalecimiento del Programa de Mejoramiento Genético de Forrajeras que se desarrolla en INIA Carillanca.
- Fortalecimiento de redes de apoyo internas y externas.

Resultados obtenidos

De acuerdo a lo planificado, la actividad permitió conocer los avances en mejoramiento genético molecular de especies forrajeras (MMSPF). **En el simposio** se comenzó con presentaciones que abordaron los objetivos actuales y beneficios del MMSPF para posteriormente en las otras sesiones conocer el avance en el desarrollo de herramientas para alcanzar tales objetivos. A continuación se destacarán los nuevos objetivos más sobresalientes y dentro de ellos las principales herramientas desarrolladas y en desarrollo.

En cuanto a los objetivos, estos son cada vez más específicos en relación a las características que se busca mejorar pero, a su vez, más integrales en cuanto a su relación con el animal, ambiente y salud humana. El mapeo, la selección asistida por marcadores y la transgenia permiten abordar objetivos cada vez más específicos y piramidar genes de interés. Por otro lado, el mejoramiento genético ya no termina con la evaluación de la producción de forraje, semilla o calidad del cultivar medido en términos convencionales (Digestibilidad, Energía, Proteína, etc.), sino que tiene un enfoque integral ya que apunta a crear cultivares y evaluar sus atributos específicos que afectan la productividad animal y la calidad composicional de los productos animales, la forma como estos productos animales satisfacen la demanda humana y afectan su salud, velando a su vez por la disminución de los impactos negativos de la producción ganadera en el ambiente.

Un ejemplo de lo anterior son los esfuerzos desplegados por grandes centros de investigación en los últimos 20 años que han permitido seleccionar variedades de ballica con mayor contenido de carbohidratos solubles. Esta tecnología determina una mejor utilización del nitrógeno de la proteína vegetal, lo que conlleva una mayor eficiencia de utilización del forraje, logrando incrementar la productividad animal y, asociado a ello, disminuyendo la fracción de nitrógeno vegetal eliminado por el animal, produciendo así una menor contaminación del medio. En cuanto a salud humana, cada vez se pone mayor énfasis en especies y variedades forrajeras que permitan producir carne y leche de mejor composición de ácidos

grasos para así lograr productos animales (carne y leche) de mejor calidad para el ser humano. El mapeo y la reciente incorporación de la selección asistida por marcadores para estas características permitirá acelerar considerablemente las ganancias genéticas que se obtendrán.

Otro ejemplo en cuanto a componentes específicos de calidad de especies forrajeras, es la actividad de la polifenol oxidasa (PPO) en trébol rosado y en algunas variedades de ballicas y el contenido de taninos de leguminosas como loteras. Tanto PPO como los taninos mejoran la digestibilidad de las proteínas ya que al formar compuestos estables con ellas, las protegen y disminuyen su degradación a nivel de rumen. Por otro lado, la PPO también forma compuestos estables con parte de los ácidos grasos insaturados del forraje, lo que determina que una menor proporción de ellos se saturen a nivel de rumen, dando lugar así a carne o leche con mayor contenido de ácidos grasos poliinsaturados que son más beneficiosos para la salud humana. En el simposio se presentaron diversos trabajos de metabólica para compuestos como los indicados, así como también para lignina y carbohidratos solubles en ballica perenne, buscando seguir la ruta metabólica de ellos, y mediante genómica buscar los genes relacionados a estas rutas y marcadores moleculares ligados.

Por otro lado, un aspecto importante es mejorar la adaptación a condiciones de estrés ambiental abiótico y biótico, como acidez de suelo, adaptación a frío y resistencia a patógenos por ejemplo. En cuanto a acidez de suelo, el mejoramiento de la adaptación se aborda buscando genes dentro de la especie y rutas metabólicas relacionadas a la expresión de dichos genes; ejemplo de ello son los genes relacionados con la secreción de ácidos orgánicos a nivel radical que confieren tolerancia a acidez. Otro enfoque plantea buscar genes en otras especies adaptadas a tales condiciones y trasferirlos posteriormente a la especie de interés (xenogenómica); tal es el caso del trabajo liderado por el DR. Germán Spangenberg del DPI, Victoria, Australia que está buscando las bases metabólicas, fisiológicas y genéticas de tolerancia a aluminio en *Microlaena stipoides* (gramínea nativa australiana) para posteriormente transferirla a gramíneas de interés forrajero. En cuanto a adaptación a frío, el mismo grupo está trabajando hace varios años en las bases metabólicas, fisiológicas y genéticas de adaptación a frío y congelamiento en *Deschampsia antarctica* Desv. Por otro lado, la resistencia a enfermedades y virus se está abordando por selección asistida por marcadores moleculares y por transformación genética. La última técnica ya ha permitido obtener líneas experimentales de trébol blanco con resistencia múltiple a virus (AMV y BYMV).

Los marcadores moleculares y el mapeo se están desarrollando ampliamente para seleccionar , entre otros, por época de floración en ballica perenne, para aumentar el contenido de carbohidratos solubles en ballica perenne, para un mayor contenido de minerales como magnesio, potasio y calcio en gramíneas, resistencia a roya de la corona en ballica y características morfológicas. Además, los marcadores moleculares se han utilizado para estudios de diversidad genética,

para optimizar colecciones de germoplasma y para seleccionar plantas madres de sintéticos. En este último aspecto se presentó un trabajo interesante en que por medio de marcadores moleculares fueron seleccionadas entre plantas destacadas de alfalfa, aquellas con mayor divergencia genética, obteniendo así cultivares sintéticos de mejor comportamiento agronómico.

Las nuevas técnicas también se están utilizando para estudiar y optimizar las relaciones huésped y simbionte o patógeno. Tal es el caso de las relaciones entre gramíneas y hongo endófito, ballica y roya de la corona y leguminosas y simbiontes fijadores de nitrógeno.

En cuanto a la transgenia, existen avances importantes en el equipo de trabajo liderado por el DR. Germán Spangenberg del DPI, Victoria, Australia. La transgenia no sólo está siendo investigada para obtener resistencia múltiple, mejorar la adaptación y calidad de las forrajeras, sino que también para mejorar el bienestar humano. Un ejemplo claro de ello es la creación de variedades de ballica cuyo polen no produce alergia en el hombre, disminuyendo así los grandes malestares que genera y los altos costos sociales del tratamiento de las alergias.

Un enfoque novedoso para mejorar la calidad de leguminosas forrajeras como trébol blanco es retardar la senescencia (envejecimiento) de sus hojas, lo que se ha logrado por transgenia. Se debatió sobre la factibilidad de liberar al mercado cultivares forrajeros transgéticos; en términos generales se considera que en especies de polinización cruzada y con presencia en la flora natural como lo son buena parte de las forrajeras, la aceptación de los transgénicos será más compleja que en especies de autopilización. Hay distintos enfoques de acuerdo a la percepción social de cada país o bloque económico. Los más escépticos a la posibilidad son los europeos, mientras que los más abiertos y avanzados en la materia son los australianos.

En términos generales y como conclusión del simposio queda claro que gracias a los esfuerzos que hoy hacen grandes centros de investigación tendremos a futuro SUPER variedades forrajeras que destacarán cada vez más por características específicas, considerando en forma integral al ambiente, al animal y a la especie humana, sin dejar de lado los requisitos fundamentales y clásicos que son rendimiento de forraje y semilla, persistencia y calidad de forraje.

El jueves 7 de julio, después del simposio, se visitó IGER. Durante la visita, participé de dos actividades muy relevantes. La primera consistió en reunirme con el Director del Depto. de producción Animal y microniología, Dr. Mike Theodorus y con algunos investigadores del departamento. En la oportunidad me interioricé y discutí de lo que están haciendo en cuanto a calidad composicional de forrajeras y su efecto sobre ambiente , animal y salud humana. Con el Dr. Theodorus se vieron dos posibilidades de interacción futura mediante proyectos a elaborar.

La segunda actividad consistió en participar de un taller para formar una red

internacional en Trifolium (ITN), la cual busca aprovechar en forma conjunta las capacidades y herramientas desarrolladas y , a futuro, abordar proyectos conjuntos. La red ITN quedó conformada y se tienen los contactos para integrarse a ella en caso de desarrollar proyectos en el área.

Resultados adicionales

Durante el simposio se aprovechó también de ver con el Dr. Germán Spangenberg los últimos detalles del documento de acuerdo entre INIA y el Department of Primary Industries Research del estado de Victoria (DPI Victoria), Australia. Dicho acuerdo fue firmado posteriormente por el Sr. Ministro de Agricultura de Chile durante la visita a Australia del Presidente de la República, Sr. Ricardo Lagos Escobar. El acuerdo establece un marco general de cooperación en áreas de genética y genómica vegetal y animal, ciencias de suelo, agua, acuicultura y alimentos. El área de contacto más avanzada entre DPI Victoria e INIA en este contexto es el mejoramiento de forrajeras con aplicaciones biotecnológicas, materia en la que se está en la fase de definición de prioridades y búsqueda de fuentes de financiamiento.

Adicionalmente, como resultado de las actividades posteriores al simposio, INIA Carillanca quedó incorporado a la red de trabajo en tréboles (International Trifolium Network, ITN).

Aplicabilidad

La aplicabilidad de los conocimientos puede abordarse desde el punto de vista de los nuevos objetivos del mejoramiento genético y de las nuevas herramientas. En cuanto a los nuevos objetivos, el enfoque más integral del mejoramiento genético desarrollado en otros países que ha dado lugar a nuevos cultivares de forrajeras que han llegado o pueden llegar a Chile, está siendo incorporado en las propuestas recientes de proyectos en la materia. Por ejemplo, las propuestas que se están elaborando para el Consorcio de la Carne contemplan la evaluación de nuevos cultivares forrajeros en temáticas como carbohidratos solubles en ballicas y la relación entre la forrajera y la calidad de la carne desde el punto de vista de ácidos grasos.

En cuanto a nuevas herramientas biotecnológicas, en años recientes se desarrolló en INIA Carillanca el proyecto FONDECYT "Desarrollo de aplicaciones biotecnológicas al fitomejoramiento de trébol rosado (Trifolium pratense L.), el cual permitió dar los primeros pasos en estas materias. Además, en INIA Carillanca

hay una buena "masa crítica" de especialistas en biotecnología que actualmente están enfocados hacia cereales y granos pero que podrían destinar parte de su tiempo o apoyar trabajos en forrajeras. Para ello, en el mediano plazo se piensa elaborar propuestas de proyectos con el apoyo del DPI Victoria, priorizando en cuanto a mejoramiento genético en Chile el mejoramiento molecular y la transformación genética en trébol rosado. En cuanto a la evaluación de materiales mejorados por DPI Victoria, se plantea la posibilidad de evaluar eventos transgénicos para resistencia a virus y senescencia retardada en trébol blanco.

Detección de nuevas oportunidades y aspectos que quedan por abordar

En Chile existe una clara falencia en mejoramiento genético de especies forrajeras. Prueba de ello es que la única especie en que utilizamos cultivares chilenos es trébol rosado, especie en la cual además de abastecer con semilla nacional al mercado interno, se destina un volumen considerable a los mercados de exportación. En todas las otras especies los cultivares utilizados son foráneos y la semilla es importada o producida en Chile pero siempre a partir de cultivares externos. Por otro lado, la reglamentación oficial que regula las evaluaciones de cultivares foráneos es poco exigente. Los dos aspectos mencionados anteriormente determinan que tanto en el mejoramiento genético de forrajeras en Chile como en la evaluación e introducción de especies y cultivares forrajeros exista una brecha tecnológica importante, lo que es especialmente relevante si se considera la incorporación al modelo agroexportador de los rubros ganaderos. Necesariamente el desarrollo de este modelo agroexportador en carne bovina y ovina y en leche bovina pasará por un mejoramiento tecnológico considerable en la producción, persistencia y atributos generales y específicos de calidad de las especies y cultivares forrajeros. Para abordar estos desafíos es fundamental la interacción con centros extranjeros que están más avanzados en las temáticas que abordó el simposio.

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3. ASPECTOS RELACIONADOS CON LA EJECUCIÓN DE LA PROPUESTA

Programa Actividades Realizadas

Nº	Fecha	Actividad	Objetivo
1	1-07- 2005 al 3 –07- 2005	Viaje Temuco-Aberystwyth	Llegar a localidad donde se realizará el Simposio
2	3-07-2005	Inscripción y Recepción.	Registrarse en Simposio y hospedaje.
3	4-07-2005	**Sesión I:** Objetivos y beneficios del mejoramiento molecular de especies forrajeras. **Sesión II:** Ligamiento, mapeo físico y clonal. **Sesión III:** Análisis QTL y disección de caracteres.	Conocer las nuevas tendencias en mejoramiento molecular de especies forrajeras y desarrollo de las herramientas mencionadas. Intercambiar opiniones con equipos de trabajo y ver posibilidades de trabajos conjuntos.
4	5-07-2005	**Sesión IV:** Genómica, especies modelo, descubrimiento de genes y análisis funcional. **Sesión V:** Uso de marcadores moleculares y bioinformática en mejoramiento. **Sesión VI:** Genética molecular y mejoramiento de "endosimbiontes" y asociaciones gramíneas/leguminosas.	Conocer los avances en genómica y uso de marcadores moleculares en mejoramiento de forrajeras. Aprender del uso de estas herramientas biotecnológicas para el mejoramiento del sistema endófito y simbiontes en asociación a mezclas gramíneas/leguminosas.
5	6-07-2005	**Sesión VII:** Transformación genética para investigación y mejoramiento. **Sesión VIII:** Diversidad genética, y sistemas de mejoramiento.	Conocer los avances en transformación genética, beneficios y evaluación de riesgos. Actualizar el conocimiento sobre la aplicación de las herramientas biotecnológicas en sistemas de mejoramiento.

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6	7-07- 2005	Visita técnica al Instituto de Investigación en Praderas y ambiente (IGER).	Participar en taller para formar red de trabajo en Trifolium (International Trifolium Network, ITN) Visitar los trabajos que están haciendo en IGER en la temática calidad composicional de especies forrajeras y su efecto sobre la calidad de carne bovina. Ver posibilidades de trabajos conjuntos con los grupos de investigación de IGER.
7	8 al 9 –07-2005	Viaje de regreso Aberystwyth, Londres, Santiago, Temuco	Regreso a actividades I&D
8		Realización de las actividades de difusión (Sección N°5)	Difundir la información entre los investigadores, productores y la industria productora y distribuidora de semilla forrajera y de prados nacional.
9	29-09-2005	Entrega de informe de viaje y actividades de difusión realizada	Informar a fuentes de financiamiento (FIA-INIA)

DOCUMENTOS

En anexos se entregan los principales documentos recopilados durante el evento, la presentación realizada en las charlas de difusión, artículo editado en la Revista del Campo Sureño, y artículo despachado para edición en revista Nuestra Tierra y Chile Agrícola.

Contactos Establecidos

En anexo se entrega el listado de todos los participantes en el simposio. En el siguiente cuadro se detalla entre estos participantes los principales contactos.

Institución Empresa Organización	Persona de Contacto	Cargo	Fono/Fax	Dirección	E-mail
IGER, UK	Dr. Michael Abberton.	Lider leguminosas forrajeras	044-1970-823000/ 044-1970-828357	Plas Gogeddan, Aberystwyth, Ceredigion SY23 3EB	michael.abberton@bbsrc.ac.uk.

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IGER, UK	Dr. Mervin Humphreys.	Director Depto. Mejoramiento genético,	044-1970-823000/ 044-1970-828357	Plas Gogeddan, Aberystwyth, Ceredigion SY23 3EB	Mervin.humphreys@bbsrc.ac.uk
IGER, UK	Dr. Michael Theodorus.	Director Depto. P.Animal, plantas y ciencias microbianas.	044-1970-823000/ 044-1970-828357	Plas Gogeddan, Aberystwyth, Ceredigion SY23 3EB	Mike.theodorou@bbsrc.ac.uk
DPI, Victoria, Australia	Dr. Germán Spangenberg.	Lider biotecnología forrajeras, DPI, Victoria, Australia	03-9479-3851/ 03-9479-3618	PBC, Agriculture Victoria, La Trobe University, Bundoora, Victoria 3083, Australia	German.Spangenberg@dpi.vic.gov.au

Material elaborado y/o recopilado

Elaborado

Tipo de material	Nombre o identificación	Preparado por	Cantidad
Difusión en charlas	Anexo 1. International Symposium on the Molecular Breeding of Forage and Turf	Fernando Ortega K.	80
Artículos divulgativos despachado a revista Campo Sureño del diario Austral (incluye versión editada), revista Nuestra Tierra y Chile Agrícola.	Anexo 2. -Eventos Internacionales sobre forrajeras: participación investigadores de INIA Carillanca -La Importancia de la Pradera.	Fernando Ortega K. y Oriella Romero Y.	1
Nómina asistentes	Anexo 3. Nómina asistentes a actividades de difusión del evento.	Fernando Ortega K. y Oriella Romero Y.	3 nóminas

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Recopilado		
Tipo de Material	**Nº Correlativo (si es necesario)**	**Caracterización (título)**
CD	Anexo 4.	Fotografías digitales de los principales posters.
Folleto	Anexo 5	High sugar grass. New varieties developed by IGER for more efficient meat and milk production
Artículo	Anexo 6	Plant-mediated lipolysis and proteolysis in red clover with different polyphenol oxidase activities
Folleto	Anexo 7	Aber Grass & Clover varieties

Programa de difusión de la actividad

En esta seccion se deben describir las actividades de difusión de la actividad, adjuntando el material preparado y/o distribuido para tal efecto.

En la realización de estas actividades, se deberán seguir los lineamientos que establece el "Instructivo de Difusión y Publicaciones" de FIA, que le será entregado junto con el instructivo y formato para la elaboración del informe técnico.

Se realiazó tres charlas de difusión de la actividad, según fechas y detalles siguientes:

19-07-2005: charla en Pitrufquen. Asistieron profesionales y agricultores de los GTT Surlat y Mune Alto. Total 27 asistentes.

20-07-2005: charla en Temuco. En el contexto de la semana del agro en la Araucanía se efectuó charla en salón de INDAP. Asisten 21 profesionales, técnicos y agricultores.

31-08-2005: Charla en Carillanca. Se realizó a 27 profesionales investigadores de INIA del área producción Animal.

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4. PARTICIPANTES DE LA PROPUESTA	
GIRAS, BECAS: Ficha de Participantes CONSULTORES: Ficha de(l) Consultor(es) EVENTOS: Ficha de Expositores y Organizadores DOCUMENTOS: Ficha de Autores y Editores	
Nombre	**Fernando**
Apellido Paterno	**Ortega**
Apellido Materno	**Klose**
RUT Personal	
Dirección, Comuna y Región	**Casilla 58-D, Temuco**
Fono y Fax	**0056-45-215706**
E-mail	**fortega@inia.cl**
Nombre de la organización, empresa o institución donde trabaja / Nombre del predio o de la sociedad en caso de ser productor	**Instituto de Investigaciones Agropecuarias, INIA carillanca**
RUT de la organización, empresa o institución donde trabaja / RUT de la sociedad agrícola o predio en caso de ser agricultor	
Cargo o actividad que desarrolla	**Director Centro Regional e investigador en mejoramiento genético de forrajeras**
Rubro, área o sector a la cual se vincula o en la que trabaja	**Forrajeras, mejoramiento genético.**

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Participantes en actividades de difusión
Es necesario registrar los antecedentes de todos los asistentes que participaron en las actividades de difusión. El listado de asistentes a cualquier actividad deberá al menos contener la siguiente información

Se adjunta listado detallado de participantes en las actividades de difusión (Anexo 3).

5. EVALUACIÓN DE LA PROPUESTA
Evaluación de la actividad para cada INICIATIVA
En esta sección se debe evaluar la actividad en cuanto a los siguientes items:
a) Efectividad de la convocatoria (cuando corresponda)
El número de asistentes a las actividades de difusión estuvo dentro de lo esperado.
b) Grado de participación de los asistentes (interés, nivel de consultas, dudas, etc)
Los asistentes a las actividades de difusión mostraron alto interés por las materias abordadas. Especialmente por las características específicas de las nuevas variedades forrajeras que se están desarrollando en otros países.
c) Nivel de conocimientos adquiridos por los participantes, en función de lo esperado (se debe indicar si la actividad contaba con algún mecanismo para medir este punto y entregar una copia de los instrumentos de evaluación aplicados)
El simposio no contemplaba un mecanismo para medir el nivel de conocimiento adquirido. La participación permitió actualizar los conocimientos en herramientas biotecnológicas aplicadas al fitomejoramiento de forrajeras.
d) Problemas presentados y sugerencias para mejorarlos en el futuro (incumplimiento de horarios, deserción de participantes, incumplimiento del programa, otros)
No hubo problemas.

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Aspectos relacionados con la postulación al programa de Captura y Difusión
a) Información recibida por parte de FIA para realizar la postulación _x__ amplia y detallada ___ aceptable ____ deficiente Justificar: Se dispuso oportunamente de las bases y formularios necesarios para postular.
b) Sistema de postulación al Programa de Formación o Promoción (según corresponda) _x__ adecuado ____ aceptable ____ deficiente Justificar: Hubo buena disposición para atender esta postulación que se realizó con los plazos bastante ajustados.
c) Apoyo de FIA en la realización de los trámites de viaje internacionales (pasajes, seguros, otros) (sólo cuando corresponda) _x__ bueno ____ regular ____ malo Justificar: Existió un buen apoyo, coordinación con el postulante y preocupación durante la ejecución (En las fechas en que se realizó el evento, ocurrieron los atentados con bomba en Londres; existió especial preocupación de la Sra. Verónica González del FIA por conocer de mi situación)
d) Recomendaciones (señalar aquellas recomendaciones que puedan aportar a mejorar los aspectos administrativos antes indicados) No se recibió oportunamente el documento "Instructivo de difusión y publicaciones FIA". A futuro se sugiere entregar junto con los otros documentos e instructivos al inicio de la actividad.

6. Conclusiones Finales de la Propuesta Completa En el caso de Giras Tecnologicas en lo posible presentar conclusiones individuales por participante
La participación en el simposio permitió captar las nuevas tendencias en mejoramiento molecular de especies forrajeras y difundirlas a productores y

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profesionales. La actualización de estos conocimientos potenciará el desarrollo e introducción de especies y cultivares forrajeros mejorados a los sistemas de producción animal de Chile.

Deseo agradecer muy sinceramente el apoyo de la Fundación para la Innovación Agraria y del Instituto de Investigaciones Agropecuarias que permitieron mi participación en el simposio.

IGER

International Symposium on the Molecular Breeding *of* Forage *and* Turf

Grassland

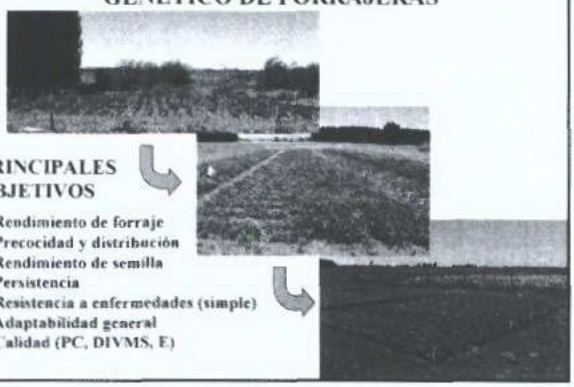
Genetic improvement of grasses and other forage crops

POST-CONGRESS SATELLITE WORKSHOP

Aberystwyth, 3rd - 7th July 2005

Fernando Ortega K. Ing. Agr. PhD.

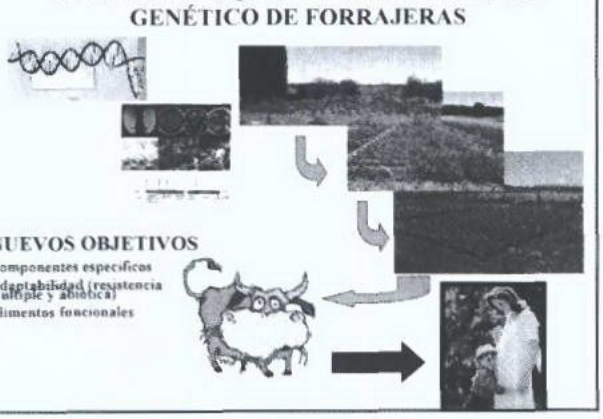
ENFOQUE CLASICO DEL MEJORAMIENTO GENÉTICO DE FORRAJERAS



PRINCIPALES OBJETIVOS

- Rendimiento de forraje
- Precocidad y distribución
- Rendimiento de semilla
- Persistencia
- Resistencia a enfermedades (simple)
- Adaptabilidad general
- Calidad (PC, DIVMS, E)

NUEVOS ENFOQUES EN MEJORAMIENTO GENÉTICO DE FORRAJERAS



NUEVOS OBJETIVOS

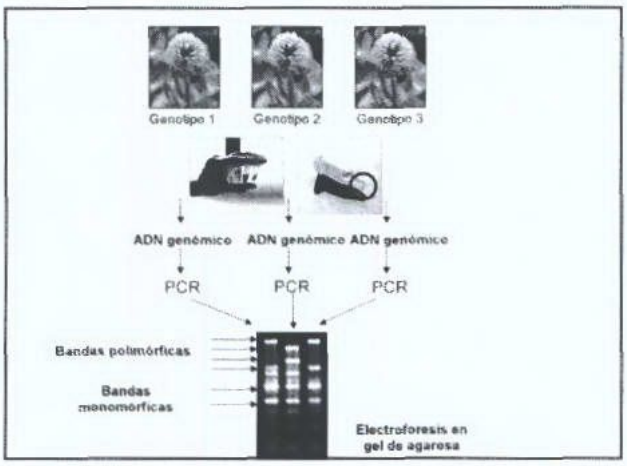
Componentes específicos

Adaptabilidad (resistencia múltiple y abiótica)

Alimentos funcionales

MARCADORES MOLECULARES

- Marcadores morfofisiológicos como marcas en las hojas (estudios flujo de genes).
- Isoenzimas detectan variaciones en formas de una misma enzimas.
- Marcadores moleculares detectan variaciones en la secuencia de bases de ADN (de RFLP a SNPs).



MARCADORES MOLECULARES

1) Mapeo & Selección asistida por marcadores.
2) Variación genética dentro y entre poblaciones, cultivares y plantas madres de sintéticos.

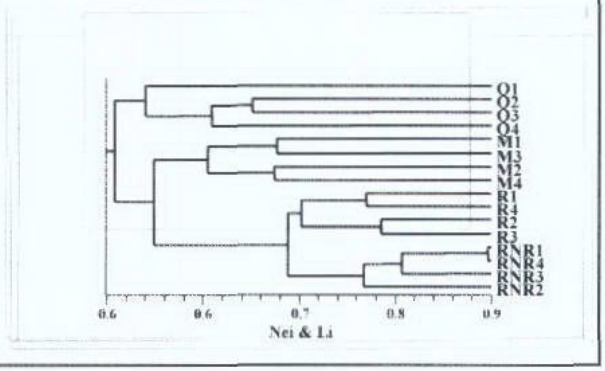
1) Mapeo & Selección asistida por marcadores

- Epoca floración en B. perenne (algunas poblaciones de mapeo, APM).
- Aumento del contenido de carbohidratos solubles en ballica perenne, fructan metabolism (APM).
- Contenido de minerales, magnecio (grass tetany), potasio-calcio, (APM).
- Resistencia a enfermedades, roya de la corona (Puccinia coronata) en BP (APM)
- Características morfológicas

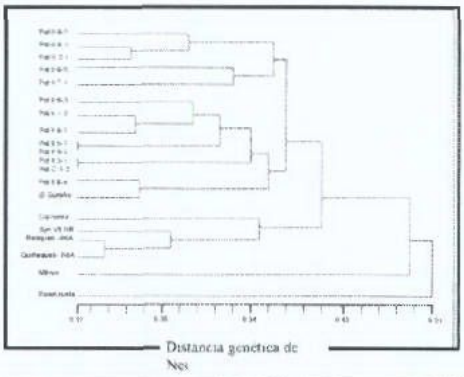
2) Variación genética dentro y entre poblaciones, cultivares y plantas madres de sintéticos

- **Diversidad genética.**
- **Identificación de variedades.**
- **Selección de plantas madres de sintéticos.**
- **Estudios taxonómicos.**
- **Colecciones CORE de germoplasma.**

Dendograma (Nei & Li) T. rosado aplicando RAPDs, INIA Carillanca



Relaciones genéticas en germoplasma de T. rosado utilizando RAPDs, INIA Carillanca



XENO-GENÓMICA

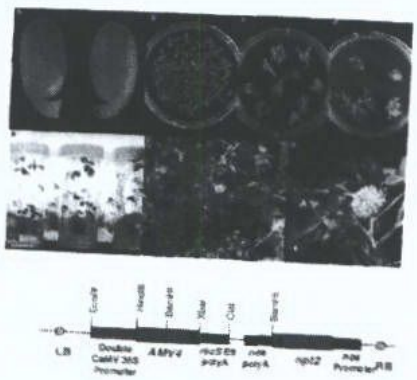
- **Estudio de genes en otras especies para posterior incorporación en la especie deseada.**
  - **Bases metabólicas, fisiológicas y genéticas de adaptación a frío y congelamiento en *Deschampsia antarctica* Desv.**
  - **Bases metabólicas, fisiológicas y genéticas de tolerancia a aluminio en *Microlaena stipoides.***

RELACIÓN HUESPED-SIMBIONTE Ó PATÓGENO

- **Estudios de la relación gramínea-endófito.**
- **Interacción ballica perenne y roya de la corona.**

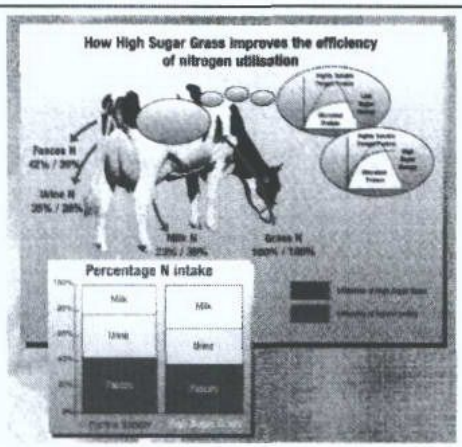
TRANSFORMACIÓN GENÉTICA

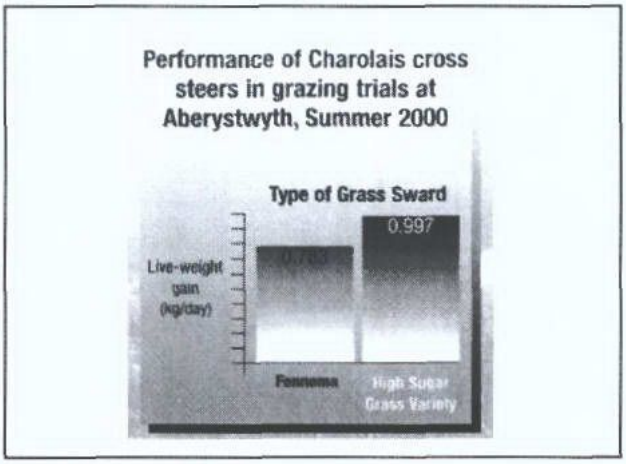
- Resistencia a virus
  - T. blanco Incorporación de resistencia a AMV, complementando resistencia natural a BYMV
- Adaptación a stress abiótico
  - T. blanco Sobreexpresión de biosintesis de acidos orgánicos para adaptación a Al alto y bajo P
  - Retardo senescencia en T. blanco

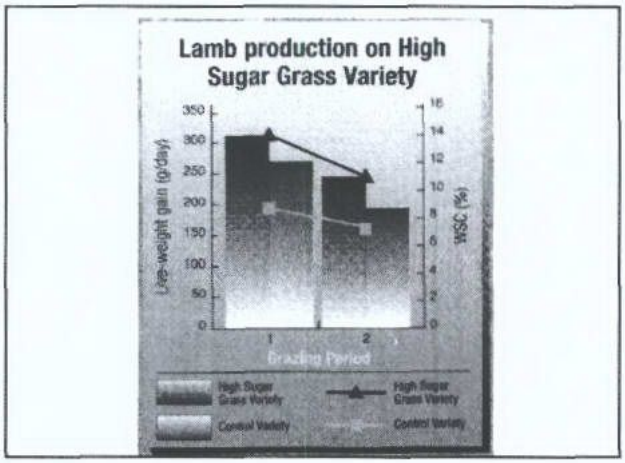


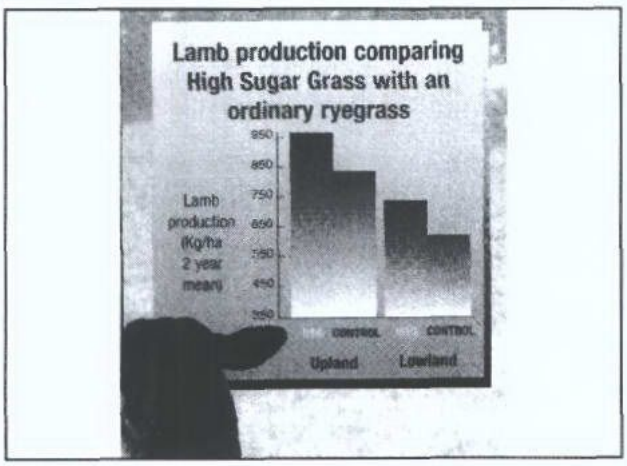
EJEMPLOS DE AVANCES EN CALIDAD ESPECÍFICA DE FORRAJERAS Y SU EFECTO EN PRODUCCIÓN ANIMAL

- **Carbohidratos solubles de gramíneas.**
- **Acidos grasos poli-insaturados (PUFA) y polifenoloxidasa (PPO)**



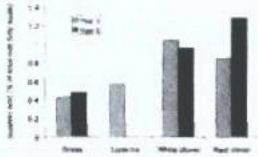






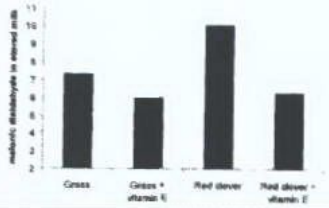
Acidos grasos poli-insaturados & relación con alimento

- Alimentación en base a praderas determina mayor contenido de PUFA en leche.
- Se acentúa en el caso de tréboles blanco y rosado, especialmente en T. rosado (PPO).

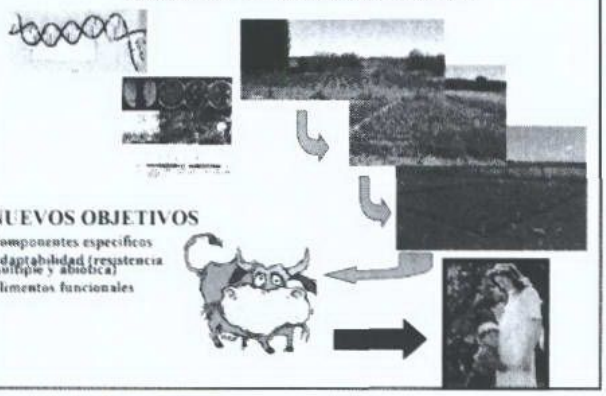


Acidos grasos poli-insaturados & relación con alimento

- **Sin embargo, mayor contenido de PUFA puede determinar reducción de estabilidad de la leche. Entonces es necesario suplementar con vitamina E.**



NUEVOS ENFOQUES EN MEJORAMIENTO GENÉTICO DE FORRAJERAS



NUEVOS OBJETIVOS

Componentes específicos

Adaptabilidad (resistencia múltiple y abiótica)

Alimentos funcionales

ANEXO 2. Artículo divulgativo despachado a Campo Sureño (incluye además el editado por la revista), revista Nuestra Tierra y Chile Agrícola

Eventos internacionales sobre forrajeras

PARTICIPAN INVESTIGADORES DE INIA CARILLANCA

Dos importantes eventos internacionales sobre forrajeras se efectuaron recientemente en Europa, donde participaron los especialistas de INIA Carillanca, Oriella Romero y Fernando Ortega. Se trata del Congreso Internacional de Forrajeras y el Simposio Internacional sobre Mejoramiento Molecular de especies Forrajeras, efectuados en Dublín (Irlanda) y Aberystwyth (Gales, Reino Unido) respectivamente. Cabe señalar que el viaje de los investigadores fue cofinanciado por la Fundación para la Innovación Agraria (FIA).

"La pradera es la base de los sistemas de producción animal y en los congresos se revisaron los últimos avances que buscan optimizar su producción, poniendo cada vez mayor énfasis en el cuidado del medio ambiente y la salud humana. En cuanto a la optimización productiva y el cuidado del medio ambiente, un ejemplo son los esfuerzos desplegados por grandes centros de investigación en los últimos 20 años que han permitido seleccionar variedades de ballica con mayor contenido de carbohidratos solubles. Esta tecnología permite una mejor utilización del nitrógeno de la proteína vegetal, lo que determina una mayor eficiencia de utilización del forraje, logrando incrementar la productividad animal y, asociado a ello, disminuyendo la fracción de nitrógeno vegetal eliminado por el animal, produciendo así una menor contaminación del medio. En cuanto a salud humana, cada vez se pone mayor énfasis en especies y variedades forrajeras que permitan producir carne y leche de mejor composición de ácidos grasos para así lograr productos animales (carne y leche) de mejor calidad para el ser humano. Los ejemplos mencionados anteriormente muestran claramente que la investigación en praderas tiene hoy un enfoque más integral, buscando no sólo mejorar la productividad animal, sino disminuir los impactos negativos en el ambiente, y por otro lado, mejorando la calidad del producto para el consumidor", señaló Fernando Ortega.

Por otro lado, los sistemas de producción en base a praderas tienen un potencial para reforzar los beneficios de los ácidos grasos en la carne y leche, mejorando la estabilidad y alterando los atributos sensoriales, cuestión que ayuda a diferenciar el producto en los mercados. Hoy más que nunca, los consumidores están conscientes de las relaciones entre dieta y salud, aspecto que ha aumentado el interés por identificar los componentes de las comidas.

"En términos generales los consumidores tienen una percepción negativa de los productos de origen animal en relación a los vegetales. En este sentido la industria debe compatibilizar los requisitos cambiantes de quienes requieren productos de origen animal de calidad, que sean seguros, saludables, trazables, diversos y convenientes. Más todavía, cuando hay evidencia creciente que el consumo de leche y carne y productos asociados puede conferir beneficios adicionales a la salud, incluso de protección contra el cáncer. La investigación en los próximos años se enfocará en manipular la composición de ácidos grasos de la leche y carne, principalmente carne, y su efecto en las características de calidad: como jugocidad, terneza y estabilidad. En este sentido, las plantas verdes son fuente primaria de omega 3 en la cadena alimenticia y es por esto que los sistemas de producción en base a praderas presentan un potencial para reforzar el volumen de ácidos grasos beneficiosos, mejorando la estabilidad de las grasas por su alto contenido de vitamina E. La carne y la leche son ricos en ácidos grasos, omega 3 y conjugados que ofrece beneficios a la salud" aclaró la Ingeniero Agrónomo, Oriella Romero.

En cuanto a las especies forrajeras existen algunas caracteristicas como la actividad de la polifenol oxidasa (PPO) en trébol rosado y en algunas variedades de ballicas que pueden reducir las pérdidas del lípido en el rumen. Así, la pradera como base de la alimentación animal, ayudaría a producir productos diferenciados para los mercados competitivos.

Otro de los temas analizados en el Congreso fueron los problemas de producción animal, relacionados con la contaminación generada a través de emisión de gases como metano,

contaminación por purines y efluentes de ensilaje. Además, aspectos de globalización, orientados al bienestar animal y los problemas medioambientales, traducido en nuevas reglamentaciones en el uso de purines y de carga animal. Esto ha reducido los márgenes de ganancia, particularmente para el productor primario.

Mejoramiento Molecular

En el Simposio fueron analizados los nuevos enfoques de mejoramiento genético en forrajeras, incorporando fuertemente nuevas herramientas biotecnológicas, que son cada vez más específicos. Cabe mencionar la modificación de componentes de las forrajeras para obtener productos animales diferenciados, los que determinan un mejor cuidado del ambiente y la salud humana. Esto podrá facilitarse aplicando técnicas moleculares para la selección y cruzamiento. Por ello se está trabajando fuertemente en mapeo de ballica, trébol blanco y otras forrajeras, a objeto de realizar selección asistida por marcadores.

Otro aspecto importante es mejorar la adaptación a condiciones de estrés ambiental, como acidez de suelo por ejemplo. Esto se aborda buscando genes dentro de la especie y rutas metabólicas relacionadas a la expresión de dichos genes a objeto de manejarla. Otro enfoque plantea buscar genes en otras especies adaptadas a tales condiciones y trasferirlos posteriormente a la especie de interés. La resistencia a enfermedades y virus se está abordando por selección asistida por marcadores moleculares y por transformación genética. La última técnica ya ha permitido obtener líneas experimentales de trébol blanco con resistencia múltiple a virus (AMV y BYMV).

"Un enfoque novedoso para mejorar la calidad de leguminosas forrajeras como trébol blanco es retardar la senescencia (envejecimiento) de sus hojas, que se ha logrado por transgenia. La transgenia no sólo está siendo investigada para obtener resistencia múltiple, mejorar la adaptación y calidad de las forrajeras, sino que también para mejorar el bienestar humano. Un ejemplo claro de ello es la creación de variedades de ballica cuyo polen no produce alergia en el hombre, disminuyendo así los grandes malestares que genera y los altos costos sociales del tratamiento de las alergias. No cabe duda entonces que gracias a los esfuerzos que hoy hacen grandes centros de investigación tendremos a futuro SUPER variedades forrajeras que destacarán cada vez más por características específicas, acompañadas de los requisitos fundamentales que son rendimiento de forraje y semilla, persistencia y calidad de forraje", dijo finalmente Fernando Ortega.

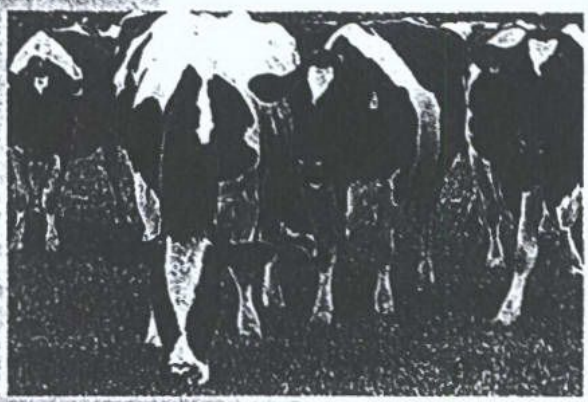
Lectura de mono:

1) Especialistas de más de 60 países participaron en elos eventos internacionales obre forrajeras.
2) La investigación en los próximos años se enfocará en manipular la composición de ácidos grasos de la leche y carne

CAMPO SUREÑO N° 1.843. Temuco, 22 de agosto de 2005. p. 3.

La importancia de La pradera

Dos importantes eventos internacionales sobre forrajeras se efectuaron recientemente en Europa, donde participaron los especialistas de Inia Carillanca, Oriella Romero y Fernando Ortega. Se trata del Congreso Internacional de Forrajeras y el Simposio Internacional sobre Mejoramiento Molecular de especies Forrajeras, efectuados en Dublín (Irlanda) y Aberystwyth (Gales, Reino Unido) respectivamente.



La pradera es la base de los sistemas de producción animal.

"La pradera es la base de los sistemas de producción animal y en los congresos se revisaron los últimos avances que buscan optimizar su producción, poniendo cada vez mayor énfasis en el cuidado del medio ambiente y la salud humana. En cuanto a la optimización productiva y el cuidado del medio ambiente, un ejemplo son los esfuerzos desplegados por grandes centros de investigación en los últimos 20 años que han permitido seleccionar variedades de ballica con mayor contenido de carbohidratos solubles. Esta tecnología permite una mejor utilización del nitrógeno de la proteína vegetal, lo que determina una mayor eficiencia de utilización del forraje, logrando incrementar la productividad animal y, asociado a ello, disminuyendo la fracción de nitrógeno vegetal eliminado por el animal, produciendo así una menor contaminación del medio. En cuanto a salud humana, cada vez se pone mayor énfasis en especies y variedades forrajeras que permitan producir carne y leche de mejor composición de ácidos grasos para así lograr productos animales (carne y leche) de mejor calidad para el ser humano, señaló Fernando Ortega.

Hoy más que nunca, los consumidores están conscientes de las relaciones entre dieta y salud, aspecto que ha aumentado el interés por identificar los componentes de las comidas.

"En términos generales los consumidores tienen una percepción negativa de los productos de origen animal en relación a los vegetales. En este sentido la industria debe compatibilizar los requisitos cambiantes de quienes requieren productos de origen animal de calidad, que sean seguros, saludables, trazables, diversos y convenientes. Más todavía, cuando hay evidencia creciente que el consumo de leche y carne y productos asociados puede conferir beneficios adicionales a la salud, incluso de protección contra el cáncer. La investigación en los próximos años se enfocará en manipular la composición de ácidos grasos de la leche y carne, principalmente carne, y su efecto en las características de calidad: como jugocidad, terneza y estabilidad", aclaró la Ingeniero Agrónomo, Oriella Romero.

REGISTRO DE ASISTENCIA A ACTIVIDAD DE DIFUSION FIA
XX INTERNACIONAL GRASSLAND CONGRESS
4th INTERNACIONAL SYMPOSIUM ON THE MOLECULAR BREEDING OF FORAGE AND TURF

LUGAR: Pitrufquén

FECHA: 19-07-05

Nombre	RUT	Dirección	Fono	Fax	Institución / Predio/ Profo	RUT Organiz.	Cargo y FIRMA	Rubro a que se dedica
Rosa Oriella Romero Yáñez		Casilla 58-D Temuco	215706	216612	INIA Carillanca		Ing. Agrónomo	Praderas
Luis H. Garin								
Luis H Garin		Donguil Rinconada						LECHERIA
René Retamal		RINCONADA DONGUIL CORREO GORBEA	92461482					LECHERIA
María Teresa Siegmund G.		Rinconada Donguil Ruta 5 Sur Km 720	4455596				M. T. Siegmund	Lechería
Jaime Gajardo C		Km 712 Ruta 5 Sur						leche
Javier Neuman		Rinconada Donguil Km 1	[illegible]					leche
Roberto González A		Rinconada Donguil Km. 6.	85987268					Leche
Hernan Herrera		6ta Faja Km 2,5	76117636					Leche
Augusto Mancini E.		Sol Quitratúe	9822360				TECNICO AGRICOLA	LECHE

REGISTRO DE ASISTENCIA A ACTIVIDAD DE DIFUSION FIA
XX INTERNACIONAL GRASSLAND CONGRESS
4th INTERNACIONAL SYMPOSIUM ON THE MOLECULAR BREEDING OF FORAGE AND TURF

LUGAR: 19 (Pilmaiquen

FECHA: 19 - Julio 2005

Nombre	RUT	Dirección	Fono	Fax	Institución / Predio/ Profo	RUT Organiz.	Cargo y FIRMA	Rubro a que se dedica
Gonzalo Müller Teiner		Fdo. San Francisco Rosal	578230		Surlat S.A.		Dueño [firma]	Lechería
Carmen Rinchalf		5ª Faja Km 14 Gorbea	94514827		Surlat		[firma]	Lechería
Gabriela Sandmeyer A.		Fdo El Encanto - R 708 Afquintue - Gorbea	1974912		Surlat		[firma]	Lechería
Emma Moënne-Loccoz		5ª Faja Km. 11	92431314		Surlat		[firma]	Lechería
Carlos Ibarra Ir		5ª Faja Km 11 Gorbea	94431311		Surlat		[firma]	Lechería
Elke v. Baer		Hijuelas Lúcas Gorbea Cas. 943	491015		Surlat		[firma]	Lechería
Eleazar Leid B		B. Encrucijada 402 Gorbea	491082		Surlat		[firma]	Lechería
René Fuentes V		Casilla Blanco del	94381837		Surlat		[firma]	Lechería
Julián Keller F		Los Aromos 1362 Temuco	92147072		Surlat		[firma]	Lechería.

REGISTRO DE ASISTENCIA A ACTIVIDAD DE DIFUSION FIA
XX INTERNACIONAL GRASSLAND CONGRESS
4th INTERNACIONAL SYMPOSIUM ON THE MOLECULAR BREEDING OF FORAGE AND TURF

LUGAR: Pitrufquén

FECHA: 19/07/05

Nombre	RUT	Dirección	Fono	Fax	Institución / Predio/ Profo	RUT Organiz.	Cargo y FIRMA	Rubro a que se dedica
Mauricio Ríos R.		Carlos Aldea 280 Pitrufquén	391067	391033	EL NACAR			LECHE
Gabriel Nuñez		GORBEA	[illegible]		LA Mariposa			Leche
Álvaro Torres		Polozuelos 220	094513000					leche.
Jorge [illegible] Hofmann		Carlos 5 Sur 707	96430896		Chumay			Tabaco
Carlos Edgardo Ulloa		Faja Ricci K 8	1974659		La Industrial		Ulloa	Leche.
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Yolanda Delgado		Ruta 5 Sur Km 706	96497353		Lugar Chumco		Yolanda Delgado	Leche
Jorge Bogdanovic		Planta Surlat Pitrufquén	391098		Surlat			Leche
Pablo López		Técnico Lechero	391098		Surlat	[illegible]		Ing. Agr.

REGISTRO DE ASISTENCIA A ACTIVIDAD DE DIFUSION FIA
XX INTERNACIONAL GRASSLAND CONGRESS
4th INTERNACIONAL SYMPOSIUM ON THE MOLECULAR BREEDING OF FORAGE AND TURF

LUGAR: Temuco. Salón Audit INDAP

FECHA: Julio 20 de 2005

Nombre	RUT	Dirección	Fono	Fax	Institución / Predio/ Profo	RUT Organiz.	Cargo y FIRMA	Rubro a que se dedica
Ingrid Holzapfel Salgado		Balmaceda 863. Pitrufquén	09-4860714 09-4881003		GTT Mune Alto	—	Ingrid Holzapfel S.	Agric.
DESIDERIO SOTO		SAUZALITO 367 P.L. CASAS	337259		INIA		[illegible]	COMUNICACIONES
[illegible] Avendaño T		[illegible]	[illegible]		INIA	—	[illegible]	[illegible]

REGISTRO DE ASISTENCIA A ACTIVIDAD DE DIFUSION FIA
XX INTERNACIONAL GRASSLAND CONGRESS
4th INTERNACIONAL SYMPOSIUM ON THE MOLECULAR BREEDING OF FORAGE AND TURF

LUGAR: Temuco, Salón Audit. INDAP

FECHA: Julio 20 de 2005

Nombre	RUT	Dirección	Fono	Fax	Institución / Predio/ Profo	RUT Organiz.	Cargo y FIRMA	Rubro a que se dedica
Alexis Luengo Zúñiga		Venecia s/N Lautaro	09-06173718		Part.	—		Agrícola en forestal
Miguel Alvarez Gómez		Villa Alegre N° 1213, Mulchén	09-4050996		Part	—		Ganadería
Jaime Santander		San Martín 836	403100		Cofra	—		Ing. agrónomo
Adrián Catalán S.		Casilla 58-D	215706		Inia	—		Ing. agrónomo
René Mondaca O.		España 201 Temuco	269647		Part	—		agricultor
Gonzalo Mondaca Sepúlveda		España 201 Temuco	269647		Part	—		Ing. agrónomo
Iván Seguel B.		G. Mackenna 477 of 103 Temuco	958500		Dpto. Frontera Conaf			
Rolando Sepúlveda Figueroa		M. Bunster 811 Angol	08400860 714121		Part	—		Veterinario
Claudio Rojas García		Casilla 58-D Temuco	215706	216-12	Ing. Agrónomo INIA Carill.	61312000-9		Ganadería

REGISTRO DE ASISTENCIA A ACTIVIDAD DE DIFUSION FIA
XX INTERNACIONAL GRASSLAND CONGRESS
4th INTERNACIONAL SYMPOSIUM ON THE MOLECULAR BREEDING OF FORAGE AND TURF

LUGAR: Temuco. Salón Audit. Ind.

FECHA: 20-Julio-2005

Nombre	RUT	Dirección	Fono	Fax	Institución / Predio/ Profo	RUT Organiz.	Cargo y FIRMA	Rubro a que se dedica
Karen [illegible] J.		[illegible]	[illegible]		Independiente		[firma]	Ing. ejec. Agrícola
Jorge Lavín C.		Paraje Exequiel Plaza 02261 Metrenco	96470757	212135	Gesag		[firma]	Ing. Agrónomo
Gonzalo Pérez M. Condo		Isabel Riquelme 02135 V. Truman Tco	733473	212135	Gesag		[firma]	Ing. agrónomo
Rubén Muñoz F.		Lautaro 67 Depto 2 Tco	92213612		estudiante		[firma]	U. católica
Luis Andrade P.								
Pablo Andrade		Fundo Paraíso Antilco Loncoche	99766067		Administrador agrícola		[firma]	manejo y engorda
Andrés Salinas P.		Freire 590 Gorbea	201935		Municipalidad de Gorbea		[firma]	Ing. agrónomo
Marcelo Prado R.		San Francisco 965 Temuco	246267		Municipalidad de Gorbea		[firma]	Ing. agrónomo
Manuel Riquelme T.		Av. Antártica 1622 Angol	715992		Municipalidad de Purén		[firma]	Ing. agrónomo

REGISTRO DE ASISTENCIA A ACTIVIDAD DE DIFUSION FIA
XX INTERNACIONAL GRASSLAND CONGRESS
4th INTERNACIONAL SYMPOSIUM ON THE MOLECULAR BREEDING OF FORAGE AND TURF

LUGAR: Carillanca

FECHA: 31-08-05

Nombre	RUT	Dirección	Fono	Fax	Institución / Predio/ Profo	RUT Organiz.	Cargo y FIRMA	Rubro a que se dedica
Carlos Ovalle		El Guano 1171	432095		INIA		Investigador	Praderas
OSVALDO TEUBER W.		LAS LENGAS 1450 COYHAIQUE	(67) 237754	"	INIA		INVESTIGADOR	FORRAJERAS
HERNAN ACUÑA		V. Mendez 515 CHILLAN	209510	209599	INIA			Investigacion Agricola
Claudio Rojas		Carrilla 58-D Tco	(45) 215796		INIA Carillanca		Cs. pg	Carne
Christian Hepp		Carrilla 296 Coyhaique	(67) 233366		INIA			Sistemas Pastoriles
Ernesto Jahn		Lo Pinto 758 Chillán	209657		INIA			Bovinos de leche
Nilo Covacevich		Croacia 961 Pta Arenas	61/710750	id	INIA			Producción Ovina
Raúl Lira F.		Angamos 1056 P. Arenas	61-710750	✓✓	INIA			Ovinos
Paz Gallardo M		Las Lengas 1450	237754	"	INIA			Ovinos

REGISTRO DE ASISTENCIA A ACTIVIDAD DE DIFUSION FIA
XX INTERNACIONAL GRASSLAND CONGRESS
4th INTERNACIONAL SYMPOSIUM ON THE MOLECULAR BREEDING OF FORAGE AND TURF

LUGAR: Cauquenes

FECHA: 31-08-05

Nombre	RUT	Dirección	Fono	Fax	Institución / Predio/ Profo	RUT Organiz.	Cargo y FIRMA	Rubro a que se dedica
Fernando Fernández		casilla 165 Cauquenes	73-512260	73-512502	INIA		Investigador [firma]	Ovinos
Sergio Iraira		Casilla 24-O Osorno	64-233515	64 237746	INIA - Remehue		Invest. Prod. Carne [firma]	Prod. de carne
Julia Avendaño		Casilla 165 Cauquenes	73-512260	idem	Rauquén	–	Investigadora Inia	Ovinos productores
Etel Latorre Pep.		Jose Mont 753 Pto Aysen	6171070	idem	INIA Kampaike	✓	Jefe Proyecto [firma]	Ovinos camélidos
Julián Parga		O'Higgins 1039 Osorno	64-233515	[tachado]	INIA-Remehue	✓	Investigador	Prod. Leche
Lidia González A.		Pedro León Ugalde Nº 1441, Santiago	(02) 5443192	-	Rayentué	"	M. Veterinario [firma]	Ovinos.
Pedro Cofré A		Casilla 426 (INIA) Chillán	42 209653	42 209599	INIA Quilamapu	"	Ing Agr [firma]	Cons Forrajes Leche
Patricio S. A O		Casilla 426 Chillán	42 209654	"	"	"	Ing Agr [firma]	Praderas
Fernando Squella N.		5 Norte 1160, Viña del Mar	32-630026	-	INIA Rayentué	"	Ing Agr [firma]	Procesos-Ovinos

REGISTRO DE ASISTENCIA A ACTIVIDAD DE DIFUSION FIA
XX INTERNACIONAL GRASSLAND CONGRESS
4th INTERNACIONAL SYMPOSIUM ON THE MOLECULAR BREEDING OF FORAGE AND TURF

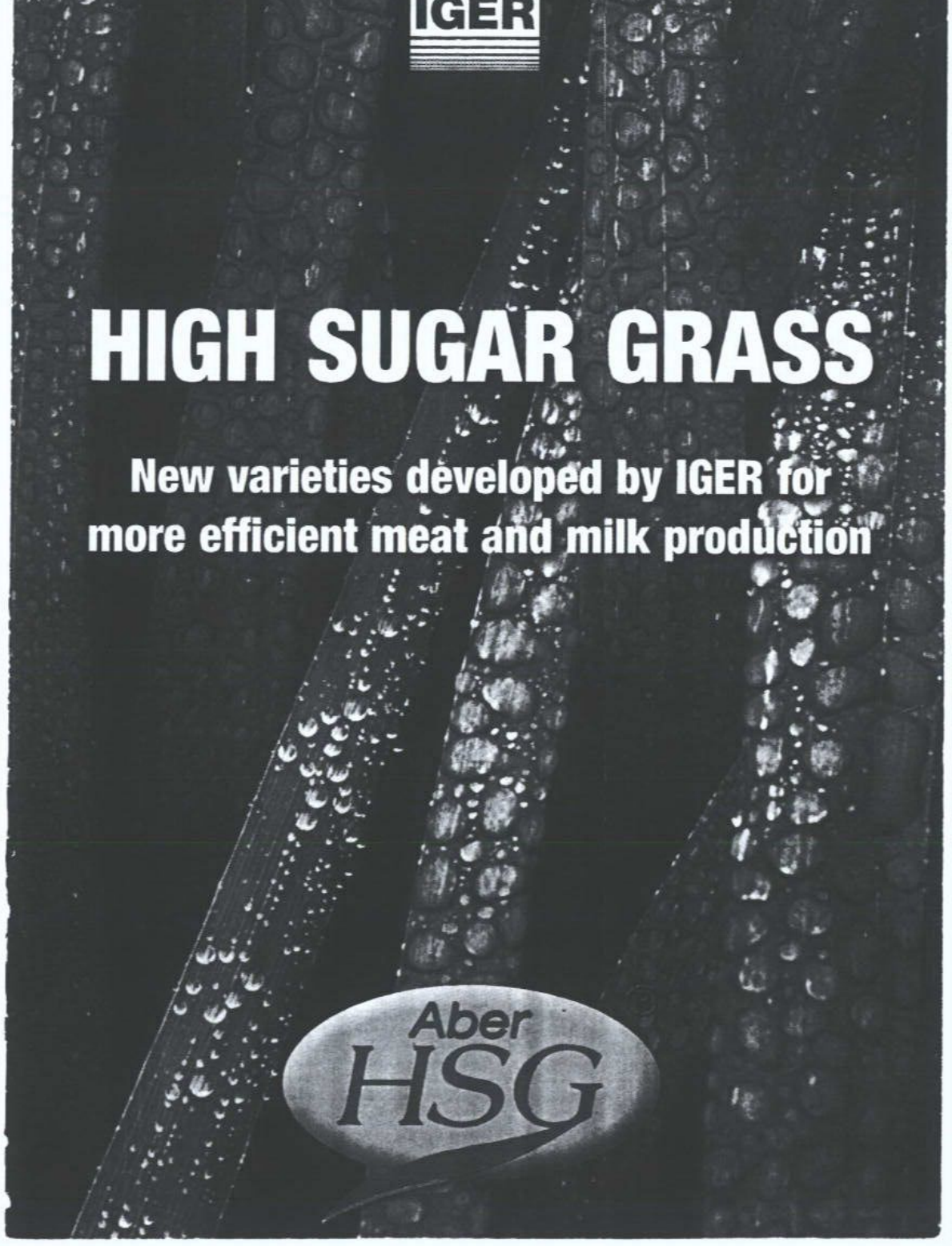
LUGAR: Carillanca

FECHA: 31-08-05

Nombre	RUT	Dirección	Fono	Fax	Institución / Predio/ Profo	RUT Organiz.	Cargo y FIRMA	Rubro a que se dedica
Alejandra Engler		Vicente Méndez 515 Chillán	209500	209593	INIA		Investigador	Leche
Victor Valencia			P-742547	13-381768	INIA		"	"
Francisco Salas		Angamos 1056 Parral	61-710150		INIA		"	Ovinos
Raúl Meneses R		Av. Matucana 5001 Coquimbo	51-243811		INIA		[firma]	
Adrian Catrileo		INIA Carillanca	215706	216112	INIA		investigador [firma]	Bovinos de carne
Hernán Felipe Elizalde		INIA Tamel Aike	233366	233366	INIA		investigador [firma]	Ovinos/Bov carne
Sergio Molina H		INIA Raihuen	381768	381768	Inia		Investigación [firma]	Bovinos de leche
Marta Alfaro		INIA Remehue	64-233515	64-237746	INIA	✓	Investigador [firma]	Contaminación ganadera
N. Teuber		✓	✓	✓	✓	✓	Investigador [firma]	Praderas

Anexo 4. Fotografías digitales de los principales posters (en CD adjunto)

# fotografía	Título poster
01	Gene associated single nucleotide polymorfism (SNP) discovery in perennial ryegrass (*Lolium perenne* L.)
02	Gene associated single nucleotide polymorfism (SNP) discovery in white clover (*Trifolium repens* L.)
03	QTL analysis of mineral content in perennial ryegrass (*Lolium perenne* L.)
04	Discovery, isolation and characterisation of promoters in white clover (*Trifolium repens* L.)
05	Gene discovery and molecular disection of fructan metabolism in perennial ryegrass (*Lolium perenne* L.)
06	Bioprospectin for genetic determinants of cold anf freezing stress tolerance in the cryophilic antartic hair grass (*Deschampsia antarctica* Desv.)
07	isolation and characterisation of genes encoding malate synthesis and transport determinants in the aluminium tolerant australian weeping-grass (*Microlaena stipoides*)
08	Production and analysis of transgenic white clover plants overexpressing organic and acid biosynthetic genes
09	Gene discovery and molecular disection of lignin biosynthesis in perennial ryegrass (*Lolium perenne* L.)
10	Microarrey-based transcriptome of the interaction between perennial ryegrass (*Lolium perenne* L.) and the fungal endophyte *Neotyphodium lolii*
11	Genetic analysis of the interaction between perennial ryegrass and the crown rust pathogen (*Puccinia coronata* f.sp *Lolii*)
12	Metabolome analysis of the interaction between perennial ryegrass (*Lolium perenne* L.) and the fungal endophyte *Neotyphodium lolii*
13	A high-throughput gene silencing approach for studying the interaction between perennial ryegrass (*Lolium perenne* L.) and the fungal endophyte *Neotyphodium lolii*
14	Marker-assisted selection for fibre concentration in Smooth bromegrass
15	Structuration of genetic diversity among and between lucerne varieties using microsatellite markers
16	A glucanase gene cosegragates with a QTL for crown rust resistance in *L. perenne*
17	Analysis of *Bromus inermis* populations using amplified fragment lenght polymorphism markers to identify duplicate accesions
18	Monitoring of gene expression profiles and identification of candidate genes involved in drought tolerance in Festuca mairei with cDNA-AFLP


IGER
HIGH SUGAR GRASS
New varieties developed by IGER for
more efficient meat and milk production
Aber
HSG

grass is the mainstay of British dairy, beef and lamb production, viewed by farmers and consumers alike as the most natural feed for ruminants.

However, agriculture has entered a new era in which efficient, home-grown forage production must go hand-in-hand with environmental considerations.

High Sugar Grass varieties, bred by the Institute of Grassland and Environmental Research, are a new generation of grasses designed to meet these needs in the 21st century.

Higher Quality Forage For A New Era Of Livestock Production

Having spent several decades enhancing the agronomic characteristics of grass - such as yield, persistency and disease resistance - plant breeders are now looking at new ways to improve herbage varieties.

At the Institute of Grassland and Environmental Research, the potential benefits of higher quality forage for sustainable livestock production were foreseen more than 20 years ago. IGER's scientists discovered that grasses with high levels of water soluble carbohydrates (sugar) could be used more efficiently by livestock. As a result, they began to develop ryegrasses with improved quality characteristics.

The first fruits of this breeding programme are High Sugar Grasses – varieties bred to contain particularly high levels of water soluble carbohydrates. Several HSG varieties developed by IGER have been rigorously tested through independent Recommended List trials and are now approved for use throughout the UK. The first HSG grass varieties and through British Seed Houses and David Bell Seeds (see back cover).

Extensive research over 20 years has now been completed to show how new HSG varieties improve performance and profitability of milk, beef and lamb production.

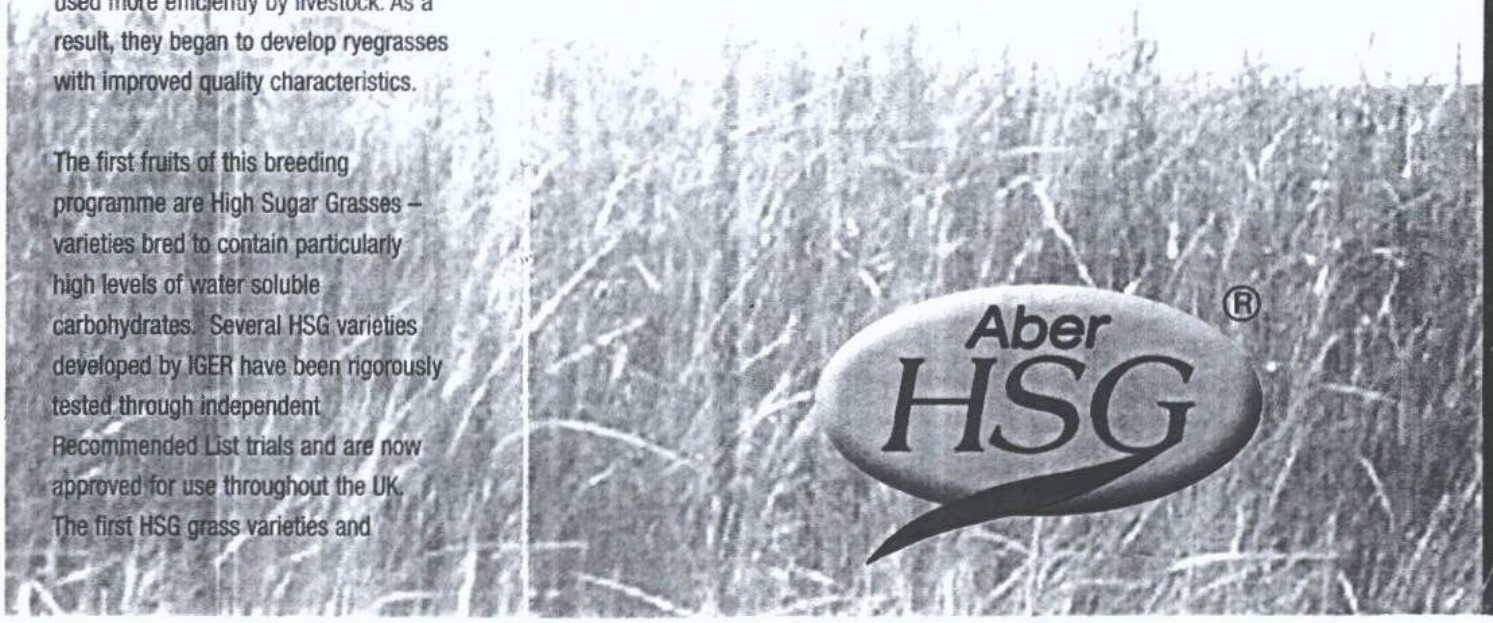
Early research with existing varieties includes:

- Grazing trials in the 1980s on six commercial dairy farms and six commercial beef farms, over two seasons;
- Sheep studies at two IGER research stations over three grazing seasons;

Recent research with improved varieties, combining good disease

- Zero grazing trials to verify the benefits of feeding high sugar grass to dairy and beef cattle;
- Nutrition studies to identify the digestive mechanism that allows ruminants to utilise HSG varieties more efficiently than recommended control grass varieties;
- Field-scale grazing trials to monitor animal performance on HSG swards;

This leaflet summarises the results of this work including initial findings from the on-going LINK sustainable livestock production project at IGER, sponsored by DEFRA, the Meat and Livestock Commission, the Milk Development Council and Germinal Holdings.



The Science Behind High Sugar Grasses

Cattle and sheep are actually poor converters of grass protein into milk and meat. When grazing ordinary grass, livestock use only about 20% of protein from the herbage for production - most of the rest is wasted in faeces and urine. This is not only financially costly, but also detrimental to the environment.

A major reason for these losses is the imbalance between readily available energy and protein within the grass. Proteins are rapidly broken down when feed enters the rumen. However, when the diet lacks readily available energy, the rumen microbes can use less of the nitrogen released from the feed, so much of it is absorbed as ammonia and eventually excreted.

Grass cell walls consist of the complex carbohydrates, cellulose, hemicellulose and lignin. Although these components can be broken down, this takes time and the carbohydrates are not, therefore, available early on, when grass protein is broken down in the rumen.

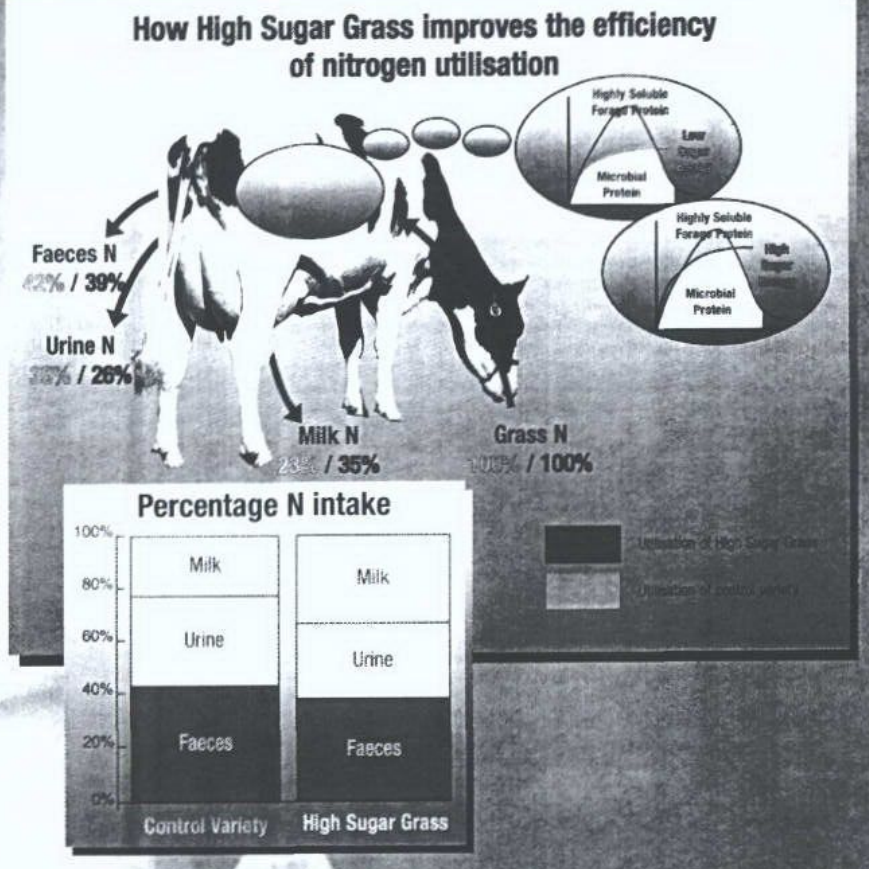
Water soluble carbohydrates in grass are the sugars found *inside* the plant cells, rather than in the cell walls themselves. They become a source of readily available energy soon after forage enters the rumen, allowing rumen microbes to process more grass protein. This protein can then be used in the production of meat and milk.

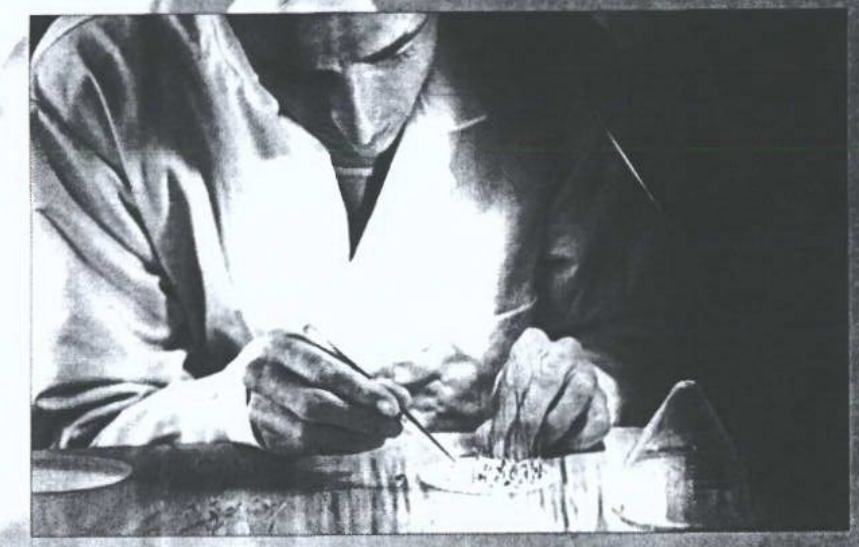
Through this mechanism, HSG varieties, with high levels of water soluble carbohydrates, can significantly improve the utilisation of protein in grass.

Research at IGER has shown that HSG varieties have consistently higher levels of sugars than standard varieties throughout the grazing season. Water soluble carbohydrate levels up to 50% higher have been recorded in some HSGs. However, studies have shown even a small difference in the level of water soluble carbohydrates can have a big effect on ruminant performance.

Several trials involving dairy and beef cattle, as well as sheep, have demonstrated significant performance benefits from feeding HSG varieties.

Results from these trials are summarised in the following sections.

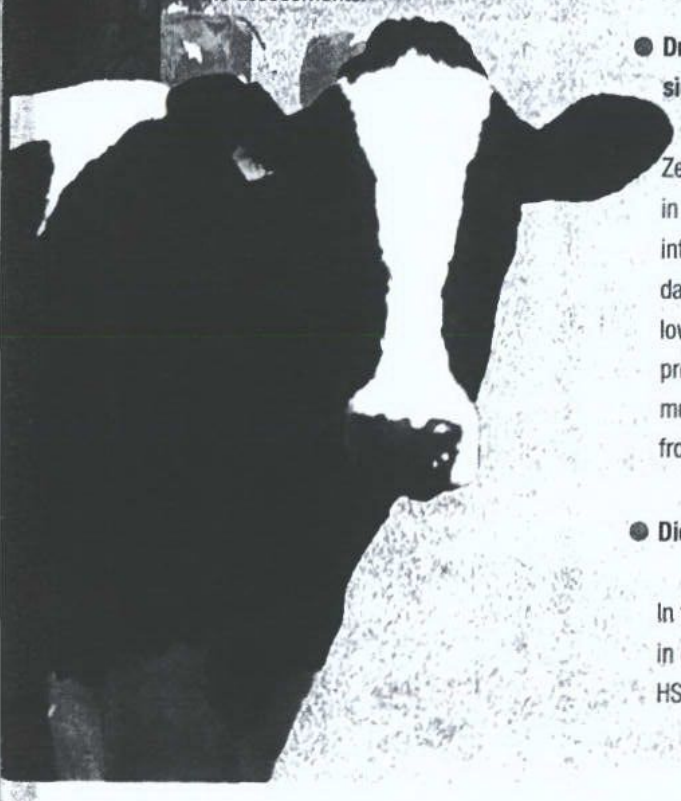




High Sugar Grass For Milk Production

Results of several studies conducted on commercial dairy farms and by IGER at its dairy unit near Aberystwyth, show that grass protein is used more efficiently for milk production when extra energy is provided by feeding HSG varieties.

Animals were fed either an experimental HSG or a recommended control ryegrass variety. Both grazing and zero-grazing techniques were used in the assessments.



The main advantages of feeding HSG varieties were found to be:

- **Milk yield increased substantially**

In an early study that looked at Italian Ryegrass across six commercial dairy farms, animals averaged 6% more milk per cow over the grazing season.

In recent zero grazing trials with Perennial Ryegrass, the average milk yield of animals fed HSG increased by 2.3kg/day in early lactation and by 2.7kg/day in late lactation, without a detrimental effect on milk quality.

- **Dry matter intakes improved significantly**

Zero grazing trials at IGER completed in 2000 found that dry matter intakes rose by around 2kg/head per day. This is particularly important in low input farming systems where producers want animals to obtain as much of their nutrients as possible from grazed grass.

- **Diet digestibility increased**

In the same trial, a 3% improvement in diet digestibility was recorded with HSG. The dry matter digestibility of the HSG variety was found to be consistently higher than the recommended control variety throughout spring, summer and autumn.

- **The amount of feed nitrogen lost in urine is significantly less**

In three zero-grazing trials involving early, mid and late lactation animals, the amount of feed nitrogen lost in the urine was reduced by up to 24% from animals fed the HSG variety. This has important implications for the environment in terms of nitrogen pollution.

6% more milk per cow over grazing season

Dry matter intakes up by 2kg/head per day

3% improvement in diet digestibility

24% less feed nitrogen lost in urine

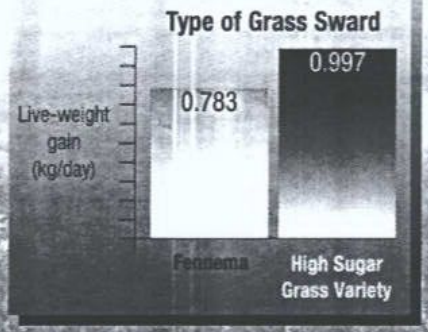
High Sugar Grass For Beef Production

Grazing trials and a companion zero-grazing study run by IGER at Aberystwyth have shown that when extra energy is provided to beef cattle by feeding HSG varieties, grass protein is used more efficiently and animal performance is enhanced.

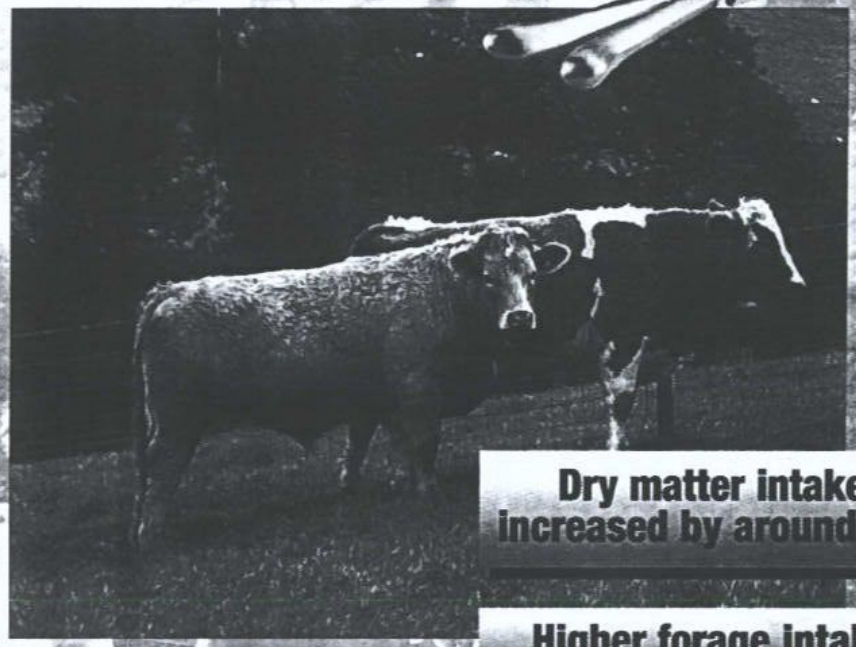
Research involved beef steers offered either an HSG variety or a recommended control ryegrass variety. No extra additional feed was given, and grass intakes and live-weight gains were monitored regularly.

- Dry matter intakes of animals fed HSG increased by around 25%, compared with those fed the control variety;
- Greater intake was achieved because the HSG variety was highly palatable. Additionally, HSG was utilised more efficiently by rumen through the rumen;
- Animals grazing HSG recorded average daily live-weight gains of 0.997kg/head per day, which was 20% higher than the gain of cattle fed the recommended control variety;

Performance of Charolais cross steers in grazing trials at Aberystwyth, Summer 2000



- In a separate zero grazing trial, animals fed an HSG variety recorded high levels of growth performance, with an average live-weight gain of 1.3kg/head per day.
- This bonus from HSG was the result of higher forage intakes and greater efficiency of grass utilisation.
- The growth rates of HSG-fed animals were enhanced, so they reached slaughter weights more quickly than those fed the control variety.



Dry matter intakes increased by around 25%

Higher forage intakes

20% higher daily live-weight gains

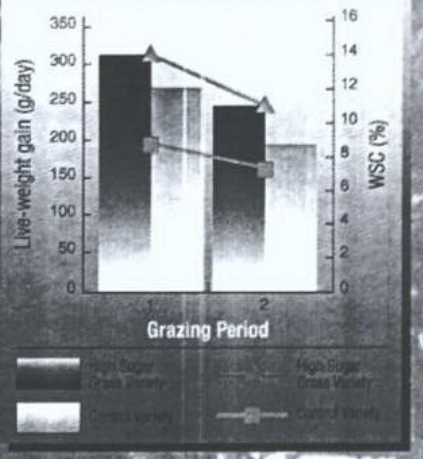
Slaughter weights reached more quickly

High Sugar Grass For Lamb Production

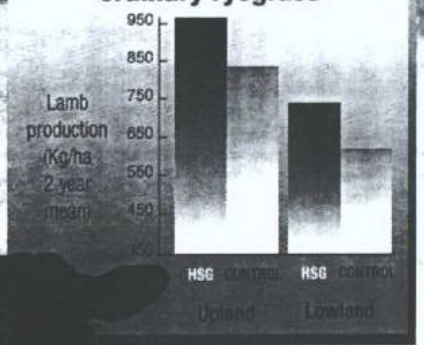
In both upland and lowland situations, IGER's grazing trials have shown HSG varieties to be superior in terms of animal performance, when compared with standard ryegrass swards.

- Initial studies on upland and lowland IGER research farms showed that an early experimental HSG variety supported significantly higher lamb growth rates;
- In recent trials with Welsh Half-bred ewes and lambs, the live-weight gain of lambs was 20% higher where animals were grazing the HSG variety;

Lamb production on High Sugar Grass Variety



Lamb production comparing High Sugar Grass with an ordinary ryegrass



- In the same study, the carrying capacity (stocking rate) of the HSG sward was 20% higher than the standard ryegrass sward;
- *Ad lib* forage intake of grazing lambs was higher on the HSG sward.

Higher forage intakes

20% higher live-weight gains

20% higher carrying capacity of HSG sward

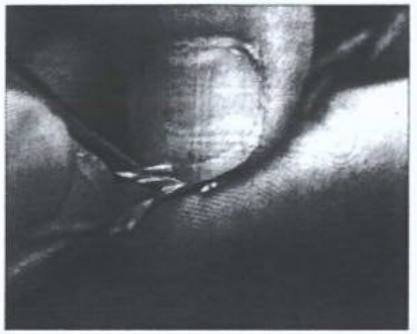
On-Going HSG Research Projects

Silage Production

Preliminary lab based trials at IGER indicate that significantly higher levels of sugar are retained in inoculated HSG silage compared with conventional varieties.

Under a new LINK sustainable livestock production project, sponsored by DEFRA, the Meat and Livestock Commission, the Milk Development Council and Germinal Holdings, IGER's scientists will evaluate the performance of dairy cattle fed silage conserved from a recommended HSG variety.

An EU funded (Sweet Grass) project involving IGER, as well as universities and research organisations in Ireland, Germany, Norway and Sweden, will also establish techniques for the conservation of HSG varieties to maximise the quantity of sugar in the ensiled crop. The effect of co-ensilage of HSGs with legumes will also be investigated.



Grazing Trials

An on-going LINK project at IGER is looking at the performance of HSG varieties throughout the grazing season. The objective of this research is to provide more evidence of the advantages of using HSG ryegrass in low-cost dairy, beef and sheep production systems. The EU funded project outlined above will also develop grazing strategies for using HSG varieties and locally adapted grasses in a range of northern European management situations.

Journal of the Science of Food and Agriculture

J Sci Food Agric **84**:1639–1645 (online: 2004)
DOI: 10.1002/jsfa.1854

Plant-mediated lipolysis and proteolysis in red clover with different polyphenol oxidase activities

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Abstract: Polyphenol oxidase (PPO) is an enzyme involved in the browning reaction of red clover leaves, when cut or crushed and exposed to air. PPO starts the browning process by oxidizing endogenous phenols to quinones, which contain electrophilic sites. These sites react with nucleophilic sites of other compounds such as proteins. The leaf tissue of two lines of red clover (cv. Milvus, a genotypic mutant with reduced PPO activity (LowPPO) and the wild-type, NormalPPO) were extracted in phosphate–citrate buffer, and a third treatment was prepared by extracting the LowPPO leaves in phosphate–citrate buffer plus 50 mM ascorbate to inhibit PPO activity (AscPPO). These extracts were compared over a 12 h time course in terms of proteolytic and lipolytic activity. Characterization of the tissues showed PPO activities of 9.11, 1.85 and 0 Δ optical density g^{-1} fresh weight min^{-1}, which were reflected in the extent of phenol (derived from quinones) binding to protein after 12 h incubation 102.3, 83.2 and 45.8 mg bound phenol g^{-1} protein ($p < 0.001$) for NormalPPO, LowPPO and AscPPO, respectively. Proteolysis measured as free amino acids released into the incubation was significantly reduced ($p < 0.001$) with increasing PPO activity, with values after the 12 h incubation of 0.03, 0.08 and 0.14 g g^{-1} protein for NormalPPO, LowPPO and AscPPO, respectively. Lipolysis, measured as the proportional decline in the membrane lipid polar fraction, was likewise reduced ($p < 0.001$) with increasing PPO activity, with values after the 12 h incubation of 0.12, 0.20 and 0.22 for NormalPPO, LowPPO and AscPPO, respectively. Changes that occurred in the lipid fractions (polar fraction, diacylglycerol, triacylglycerol and free fatty acids) during the incubations are also reported and discussed. These results support the selection of forages high in PPO activity to reduce protein and lipid loses *in silo* and potentially in the rumen.

Keywords: lipolysis; proteolysis; polyphenol oxidase; senescence; plant enzymes

INTRODUCTION

The enzyme polyphenol oxidase (PPO) is a copper metalloprotein involved in a number of browning reactions in plants, including that of red clover leaves when cut or crushed and exposed to the air. PPO catalyses the oxidation of endogenous phenols to quinones in the presence of oxygen.[1] The PPO-generated quinones are highly reactive, electrophilic molecules which covalently modify and crosslink a variety of nucleophilic cellular constituents, such as proteins, amines and amides, leading to the formation of melanin pigments.[2] It has been shown that this browning reaction is associated with a reduction in the extent of red clover proteolysis both *in silo*[3] and in the rumen.[4] This could be due to the complexing of leaf proteins[5] and/or the denaturing of plant proteases.[6] The reduction in protein breakdown through this mechanism results in over 80% of red clover silage protein being retained as true protein, resulting in improved N-use efficiency and final product quality.[7] If lipolytic activity is also reduced by this PPO-induced chemical cascade then it may result in a significant reduction in lipolysis both *in silo* and *in vivo*. Lipolysis (the splitting of the ester bond in glycerol based lipids) is a prerequisite for the microbial hydrogenation (biohydrogenation) of unsaturated fatty acids. This addition of hydrogen to unsaturated fatty acids in the rumen is thought to increase the saturated fat content of ruminant products and is considered to reduce the healthiness of ruminant fat.[8] Such a reduction in ruminal hydrogenation was observed in steers on red clover silage diets[9] and this has been followed through into the enhanced polyunsaturated nature of the product, in milk and beef muscle.[10] This effect may be due to the action of PPO reducing plant lipolysis as well as proteolysis. If this is confirmed then supplementing ruminant diets with red clover may improve the healthiness of the ruminant products. This

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experiment aims to compare the extent of proteolysis and lipolysis in extracts of red clover with: (a) a normal activity of PPO (NormalPPO), (b) a mutant red clover line with a low activity of PPO (LowPPO) and (c) this mutant line treated with ascorbate to inhibit PPO activity (AscPPO).

MATERIALS AND METHODS

Leaf extraction and incubation

The red clover (*Trifolium pratense* L) plants used were clonal lines of two plants of cv. Milvus which were selected on the basis of high or low PPO activity. These were grown under controlled conditions (20–15 °C; day and night temperatures with 16 h supplemental light, starting at dawn). They were cut back at regular intervals and were approximately 8 weeks into a regrowth period prior to the start of the experiment. Ninety grams (fresh weight) of leaf tissue were harvested from both normal PPO red clover and the low PPO red clover plants, frozen in liquid nitrogen and each batch extracted by blending in 360 ml of phosphate/citrate buffer (McIlvaine) pH 7 over a 10 min period. A further 90 g sample of leaf tissue from the low PPO red clover was extracted with 50 mM ascorbate in the buffer, giving three red clover extracts: NormalPPO, LowPPO and AscPPO. Each extract was then sub-sampled into tightly stoppered incubation bottles, in 20 ml aliquots. Three replicate bottles were allocated to one of six sampling points of a 12 h time scale (0, 1, 2, 4, 6 and 12 h), giving 18 bottles per treatment. The initial (0 h) replicate time points were then destructively sampled and the remaining extracts were left at room temperature (ca 20 °C). The time taken from tissue collection to the initial time point being taken was ca 20 min.

Harvesting, enzyme deactivation and lipid extraction

At each time point the appropriate incubation bottles were removed and a 1 ml sample of the incubation medium taken and frozen (−20 °C) for later analysis of free amino acids and protein-bound phenol to determine the extent of proteolysis and the degree of PPO activity, respectively. The remaining bottle contents were treated with 25 ml of isopropanol–chloroform (1:1 v/v) plus 1 ml of internal standard (2.5 mg C19:0 ml^{-1} chloroform). Isopropanol was used as a lipid extraction solvent as it has been shown to be extremely rapid in deactivating lipases.[11] This mixture was blended and left overnight to extract at 4 °C during which time a gelatinous precipitate formed. The precipitate was dissolved in 50 ml of isopropanol–chloroform to form an approximate ratio of (4:1; (isopropanol–chloroform):(aqueous buffer + endogenous water)). The extraction was then filtered through a sintered glass Buchner funnel (medium porosity) fitted with a ball joint for use with pressure. The residue was washed with 5 ml of isopropanol–chloroform, allowing to soak for 2 min before reapplying pressure. The filtrate was centrifuged at 900 × *g* and the aqueous top layer removed. Twenty millilitres of saline (0.02% $CaCl_2$, 0.017% $MgCl_2$, 0.29% NaCl, 0.37% KCl) were added, again to produce the 4:1 ratio of (isopropanol–chloroform): saline, and centrifuged as before. The aqueous layer was again discarded and the remaining bottom layer rotary evaporated to dryness at 50 °C with 5 ml of isopropanol (to form an azeotrope with any remaining water). The dry residue was then resuspended in 10 ml of chloroform–methanol (2:1 v/v).

PPO activity assay, free amino acid and bound phenols determination

For the PPO activity assay plants were extracted according to the method of Winters *et al.*[12] and assayed according to the method of Robert *et al.*[13] Samples taken for analysis of protein fractions were centrifuged at 15 000 × *g* for 10 min at 4 °C and the supernatant retained. Soluble protein was precipitated by addition of 1 ml 20% trichloroacetic acid, 0.4% phosphotungstic acid. Following incubation for 30 min at 0 °C, precipitated protein was centrifuged at 15 000 × *g* for 10 min at 4 °C and the supernatant retained for analysis of free amino acid content according to the method of Winters *et al.*[14] The protein pellet, which included bound phenol, was dissolved in 6 ml 0.1 M NaOH and analysed for protein and phenol content according to the method of Potty.[15] Protein concentration was based on the difference in response using Folin's reagent with and without copper. The assay response without copper is mainly due to bound phenol content, whilst the response with copper is due to a combination of protein and bound phenol content. By estimating the protein component of this response it was possible to calculate the concentration of bound phenol.

Lipid fractionation and analysis

A 5 ml aliquot of the chloroform–methanol lipid extract was taken for lipid fractionation by thin layer chromatography as described by Nichols.[16] The lipid fractions (polar fraction, diacylglycerol, triacylglycerol and free fatty acids) and remaining total lipid samples were converted to methyl esters using the bimethylation procedure of Kramer and Zhou[17] and analysed by gas liquid chromatography on a CP Sil 88 FAME column (100 m × 0.25 mm I.D., Chrompack UK Ltd, London, UK) with split injection. Peaks were identified from standards and quantified using the internal standard (C19:0). Lipolysis was calculated by expressing the decrease in fatty acid content of the polar fraction between the initial time point T_0 and incubation time point T_x, as a proportion of total fatty acid content (polar fraction + triacylglycerol + free fatty acids + diacylglycerol) at T_0. Lipolysis was analysed using a repeated measures analysis of variance using Genstat 5: Lawes Agricultural Trust (1997). Changes in the free fatty acid fraction and the release of free amino acids were determined to be linear and were

described by linear equations using Genstat 5: Lawes Agricultural Trust (1997). The changes in the polar and diacylglycerol lipid fractions were calculated using a nonlinear regression analysis in Genstat 5: Lawes Agricultural Trust (1997) where lipid fractions were the response variate and time the explanatory, with the rate of decline as the parameter in the exponent.

RESULTS AND DISCUSSION

Polyphenol oxidase activity and proteolysis

The activity of polyphenol oxidase in the red clover extracts was characterised to be 9.11, 1.85 and 0 Δ optical density g^{-1} fresh weight min^{-1} for NormalPPO, LowPPO and AscPPO, which is reflected in the concentration of bound phenols (derived from quinones) in the extract medium (Fig 1). The final concentration (12 h) of bound phenols was significantly different between the three treatments at 102.3, 83.2 and 45.8 mg g^{-1} protein for NormalPPO, LowPPO and AscPPO, respectively. This indicates that the activity of PPO was, as expected, highest for the NormalPPO and lowest for the LowPPO line treated with ascorbate to inhibit PPO activity, ie AscPPO. The initial high level of bound phenols in the NormalPPO treatment indicates that the reaction was extremely rapid and mainly took place prior to the initial sample being taken, with consequent exhaustion of the phenol substrate. In addition, PPO activity can be inhibited by the quinone products or limited by a low O_2 supply.[2] It must also be noted that bound phenols are expressed on a protein basis. Protein breakdown occurred during the incubation period and more probably in phenol-free protein fractions. This implies that some increases in protein-bound phenol content are possibly due to protein breakdown, particularly with the LowPPO and AscPPO treatments, therefore the level of bound phenol in these incubations would be overestimated.

The increase in free amino acids in the incubation medium as a measure of plant-mediated proteolysis is shown in Fig 2. Zhu *et al*[18] provided

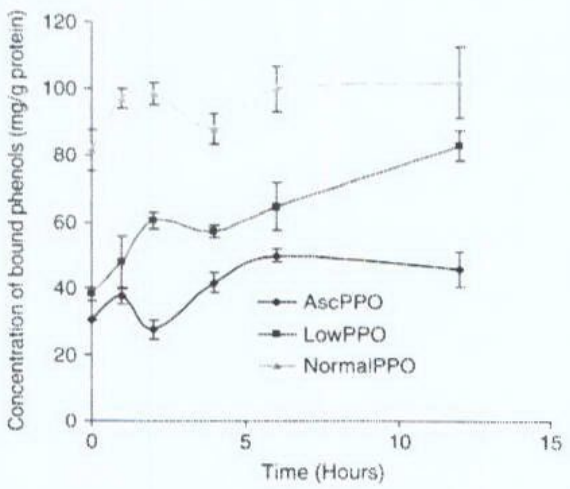

Figure 1. Concentration of bound phenols (derived from quinones) in the incubation medium.

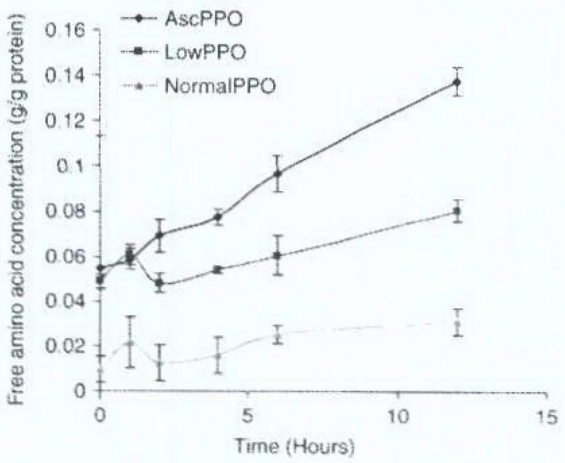

Figure 2. The release of free amino acids into the incubation medium as a measure of plant-mediated proteolysis.

evidence for the role of plant proteases in the degradation of herbage proteins, in rumen-like conditions. In the present experiment there was a linear increase in the concentration of free amino acids in the AscPPO treatment, with the line of best fit described by: $FAA = 0.053(\pm 1.2 \times 10^{-3}) + 0.007(\pm 1.0 \times 10^{-4})t$ ($r^2 = 0.97$; $p < 0.001$, where FAA = free amino acid in g g protein^{-1} and t = time in hours); however there was no significant rise in the other two treatments. These results agree with the findings of Jones *et al*,[6] who showed a 40% reduction in proteolysis with red clover silage compared with alfalfa silage, 7 days after ensiling. They related this reduction in proteolytic activity to the polyphenol oxidase content of the red clover. In this experiment there was a high negative correlation ($r = -0.79$; $p < 0.05$) between polyphenol oxidase activity and proteolysis (estimated by free amino acid concentration). This suggests that increasing the extent of PPO activity decreases proteolytic activity. This was clearly demonstrated with the AscPPO treatment and, although there was no significant linear increase in free amino acid concentration for the LowPPO and NormalPPO treatments, the LowPPO treatment had a significantly higher concentration of bound phenols (0.08 vs 0.03 g g^{-1} protein; $p < 0.001$) and a larger increase of free amino acids during the 12 h incubation period (2.6×10^{-3} vs 1.7×10^{-3} g g^{-1} protein h^{-1}; $p < 0.05$) than the NormalPPO treatment, suggesting an increase in proteolysis with reduced PPO activity. The level of free amino acids for the NormalPPO at the initial time point (0 h) (ca 1.9 mg g^{-1} fresh weight; FW) is probably a reflection of normal free amino acid levels in red clover, calculated to be ca 2.2 mg g^{-1} FW.[19] The differences between NormalPPO and LowPPO and AscPPO in free amino acid concentration at time 0 h probably reflects rapid proteolysis during the preparation period.

Lipolysis and changes in the lipid fractions

The extent of lipolytic activity at time T_x is shown in Table 1 and the changes that occurred in the different

Table 1. Extent of lipolytic activity in the three red clover treatment incubations (AscPPO, LowPPO and NormalPPO) at time T_x

	Incubation time (T_x) (h)				
Lipolysis (%)	1	2	4	6	12
AscPPO	4.00[b]	8.99[b]	12.9[b]	15.3[b]	21.6[b]
LowPPO	3.97[b]	5.42[a]	9.38[a]	14.1[b]	20.0[b]
NormalPPO	1.87[a]	5.41[a]	8.59[a]	11.6[a]	11.9[a]
SED	0.551	0.671	0.295	1.05	1.76
Significance	**	***	**	*	**

Numbers followed by the same letter are not significantly different within each column. SED, standard error of the difference; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

lipid fractions are shown in Fig 3(a–d). The degree of lipolysis, measured as the percentage decline in the fatty acid content of the membrane phospholipids and galactolipids (polar fraction), was significantly lower in the NormalPPO extraction compared with the AscPPO extraction across all time points ($p < 0.05$) with the LowPPO extraction being intermediary between the two. Lee *et al*[20] noted that red clover had a significantly lower lipolytic activity than perennial ryegrass. They suggested that this reduction may be due to the PPO activity of the red clover denaturing lipases in a similar way to its postulated denaturation of proteases.[6] The results of this experiment agree with their findings. However we postulate that the decline in lipolytic activity may also be due to the quinones, produced from the PPO chemical cascade, binding with the nucleophilic sites of the polar lipids.[21] This may offer some level of protection from lipase activity and so reduce lipolysis.

Four groups of lipid-degrading enzymes, phospholipases, phosphatases, lipolytic acyl hydrolase and lipoxygenases are associated with microsomal, mitochondrial, chloroplastic and cell membrane turn over.[22] They are activated at the onset of tissue senescence, which may be induced in many natural plant processes, such as fruit, flower and leaf abscission. It may also be induced by mechanical or heat damage, where these enzymes, which would otherwise be physically separated from the substrates, will be in direct contact with various cytoplasmic organelles and the cell lipid bilayer.[23] Lee *et al*[24] demonstrated *in vitro* that plant-mediated lipolysis was induced in an environment, which simulated the mechanical breakdown of plant cells by chewing and ruminating and the temperature stress of the rumen (39 °C). The loss of membrane phospholipid (the polar fraction) is one of the best documented indices of membrane lipid metabolism[25] and this is clearly seen in Fig 3(a). The curves for the three treatments are exponential and are described by the equations in Table 2. The rates of decline in the polar fraction of the LowPPO and AscPPO treatments were similar and both were significantly higher ($p < 0.001$) than that of the NormalPPO treatment. This highlights the relationship between reduced lipolytic membrane breakdown and PPO activity.

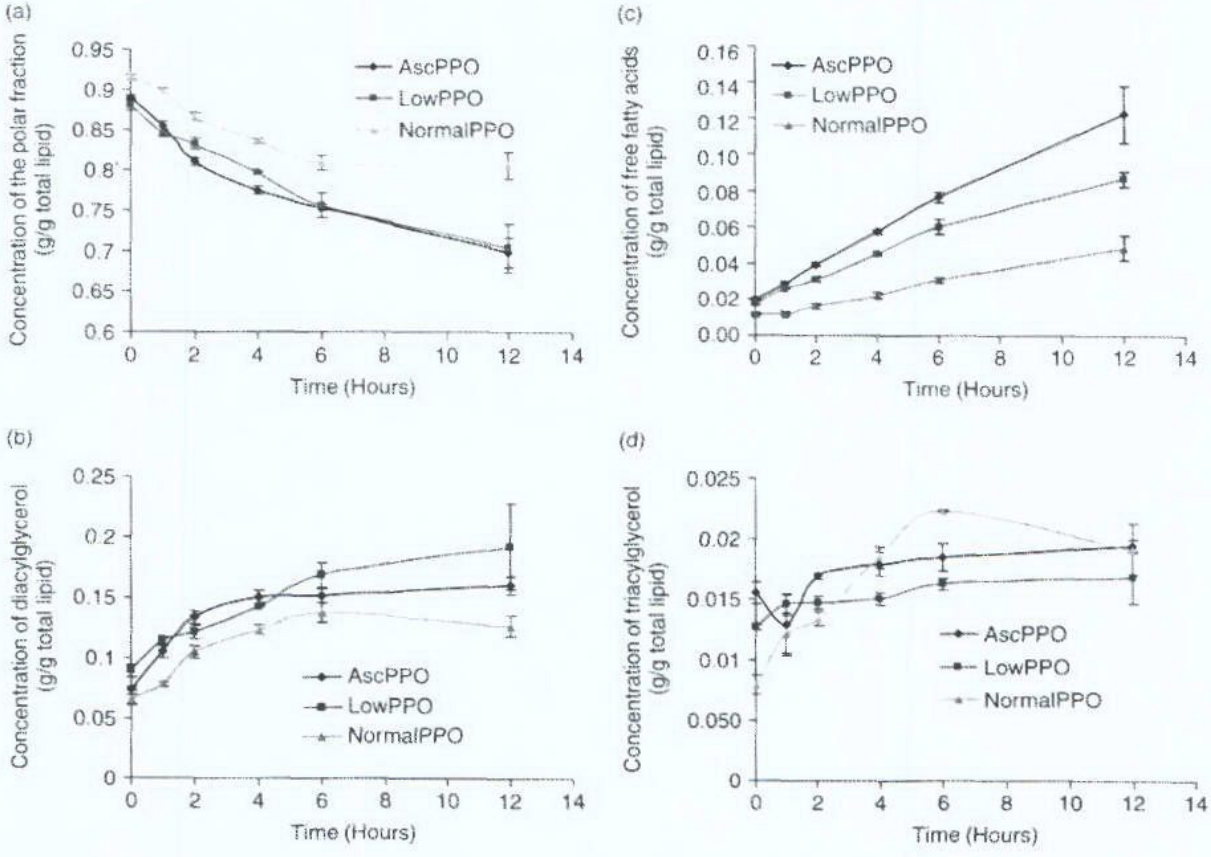

Figure 3. Changes in the lipid fractions; (a) Polar fraction; (b) diacylglycerols; (c) free fatty acids; and (d) triacylglycerol.

Table 2. Linear and nonlinear regression equations to describe the changes in lipid fractions during the 12 h incubation period

Lipid fraction	Treatment	Regression equation for line of best fit (±SE)	Significance	
			Linear	Exponential
PF	NormalPPO	$PF = 0.80(\pm0.01) + 0.12(\pm0.01)\ e^{-0.74(\pm0.066)t}$	NS	***
	LowPPO	$PF = 0.65(\pm0.05) + 0.23(\pm0.04)\ e^{-0.89(\pm0.037)t}$	NS	***
	AscPPO	$PF = 0.68(\pm0.02) + 0.21(\pm0.02)\ e^{-0.82(\pm0.037)t}$	NS	***
DG	NormalPPO	$DG = 0.13(\pm0.01) - 0.07(\pm0.01)\ e^{-0.64(\pm0.115)t}$	NS	c***
	LowPPO	$DG = 0.21(\pm0.03) - 0.12(\pm0.02)\ e^{-0.86(\pm0.061)t}$	*	c**
	AscPPO	$DG = 0.16(\pm0.01) - 0.09(\pm0.01)\ e^{-0.58(\pm0.101)t}$	NS	c***
FFA	NormalPPO	$FFA = 0.01(\pm0.001) + 0.003(\pm0.0001)t (r^2 = 0.92)$	***	NS
	LowPPO	$FFA = 0.02(\pm0.001) + 0.006(\pm0.0003)t (r^2 = 0.97)$	***	NS
	AscPPO	$FFA = 0.02(\pm0.002) + 0.009(\pm0.0004)t (r^2 = 0.91)$	***	NS

PF, polar fraction; DG, diacylglycerol; FFA, free fatty acid; TAG, triacylglycerol; *t*, time (h); c, critical exponential. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The breakdown of the polar fraction occurs through two main mechanisms: (a) phospholipases cleave phospholipids at the terminal phosphodiesteric bond which is then acted upon by the appropriate phosphatase, depending on the nature of the polar head group (eg phosphatidic acid phosphatase), resulting in the formation of a diacylglycerol. This is then further degraded into free fatty acids and glycerol by lipolytic acyl hydrolase.[26] (b) The specificity of the enzyme lipolytic acyl hydrolase is low and as such it will lypolyse phospholipids directly without the formation of the diacylglycerol intermediate.[27] The first mechanism of polar fraction degradation would explain the rise in the diacylglycerol fractions of all three treatments shown in Fig 3(b). The three treatments behaved similarly with a critical exponential increase in the diacylglycerol fractions over time; these increases are described by the equations given in Table 2. Although there are no significant differences between the rates of diacylglycerol formation, from these equations, there was a significantly lower concentration of diacylglycerol after 12 h with the NormalPPO treatment, again suggesting a lower lipolytic activity than with the other two treatments (0.12, 0.19 and 0.16 g diacylglycerol g^{-1} total lipid for NormalPPO, LowPPO and AscPPO, respectively).

Both described mechanisms for the breakdown of the polar fraction would explain the rise in free fatty acids shown in Fig 3(c). This is the clearest evidence for a difference in lipolytic activity between the three treatments as there is an inverse relationship between levels of free fatty acids and PPO activity. The linear increases in free fatty acid concentration are described by the linear regression equations given in Table 2. This is in agreement with the findings of Lee *et al*[20] who observed a significant critical exponential increase in diacylglycerol and a linear increase in free fatty acids for both red clover and ryegrass, which coincided with a decline in the polar fraction during plant-mediated lipolysis. However, they also showed a critical exponential increase in triacylglycerol (TAG) concentration during lipolysis. In the present study there was a trend ($p < 0.1$) for increased TAG up to 6 h with the NormalPPO treatment and throughout the 12 h incubation period on the LowPPO treatment but these were not significant (Fig 3(d)), possibly as the result of the low levels of TAG found in the samples (0.008–0.02 g TAG g^{-1} total lipid). Browse *et al*[28] and Sakaki *et al*[29,30] highlighted a mechanism for an increase in TAG in leaf tissue during periods of stress (such as being eaten). TAG in leaves is usually found in very small amounts, but an enzyme acylCoA:*sn*-1,2-diacylglycerol acyltransferase (DAGAT), which is found mainly in the envelope membrane of chloroplasts, up-regulates during periods of stress and converts diacylglycerols released by degradation of the membrane lipid bilayer into TAG.

Fobel *et al*[31] suggested that there was a selective depletion of polyunsaturated fatty acids from membranes during senescence and an ensuing decrease in the polyunsaturated (PUFA) to saturated (SFA) fatty acid ratio of the polar fraction. However in this study the PUFA:SFA ratio of the polar fraction did not change significantly with time or across treatments with a mean value of 6.10. This suggests that induced lipolysis through an imposed stress may result in a different pattern of membrane lipid degradation than natural senescence. In addition during natural senescence, released free polyunsaturated fatty acids, such as linoleic and linolenic acids, would be converted into hydroperoxy derivatives by lipoxygenases. These in turn would be converted into one of 3 products; aldehydes by hydroperoxide lyase, jasmonic acid and methyl jasmonante by allene oxide synthase, or epoxides by peroxygenase, all of which can be recycled by the plant.[32,33] All of these reactions would be prevented by the absence of oxygen, such as in anaerobic *in vitro* incubations and the rumen of grazing ruminants. This is borne out by the results in Table 3, which show no change in the concentration of linoleic and linolenic acids between the initial time point (0 h) and the end of the incubations (12 h) across all three treatments. This is in contrast to the results of Dewhurst and King,[34] which showed a significant decline in the concentration of these major fatty acids during an aerobic wilt prior to silage making.

The net result of the depletion in membrane phospholipid, whether through natural or induced lipolysis, is a decrease in bulk membrane lipid fluidity which may alter the conformation of membrane

Table 3. Changes in the concentration (g g^{-1} total fatty acids) of the three major fatty acids between the initial time point (0 h) and end point (12 h) of the three red clover treatments

	NormalPPO		LowPPO		AscPPO		Treatment	Significance	
	0 h	12 h	0 h	12 h	0 h	12 h	SED	Time	Treatment
C16:0	0.127	0.123	0.133	0.137	0.120	0.130	0.0039	NS	**
C18:2	0.140	0.140	0.141	0.142	0.130	0.133	0.0015	NS	NS
C18:3	0.640	0.643	0.613	0.603	0.633	0.623	0.0054	NS	***

C16:0, palmitic acid; C18:2, linoleic acid; C18:3, linolenic acid. ** $p < 0.01$; *** $p < 0.001$.

proteins, rendering them prone to proteolysis,[25] either plant-mediated or microbial when ingested by ruminants. Therefore high PPO activity may infer a further protective method for reduced proteolysis by reducing the destruction of the lipid bilayer.

CONCLUSIONS AND FUTURE STUDIES

The results of this experiment have highlighted a link between reduced proteolysis and lipolysis in red clover extracts with increasing PPO activity. This may have consequences *in silo* as protein and polyunsaturated fatty acid losses could be further reduced by selecting forages with high PPO activities. Future studies will investigate whether lipid protection is due to the denaturation of plant lipases or through quinone binding to polar lipids. The role of red clover PPO in lipid protection during grazing and ingestion into the anaerobic rumen also needs to be determined. A reduction in lipolysis in the rumen would result in lower levels of polyunsaturated fatty acid biohydrogenation and therefore increase the PUFA:SFA ratio of ruminant products.

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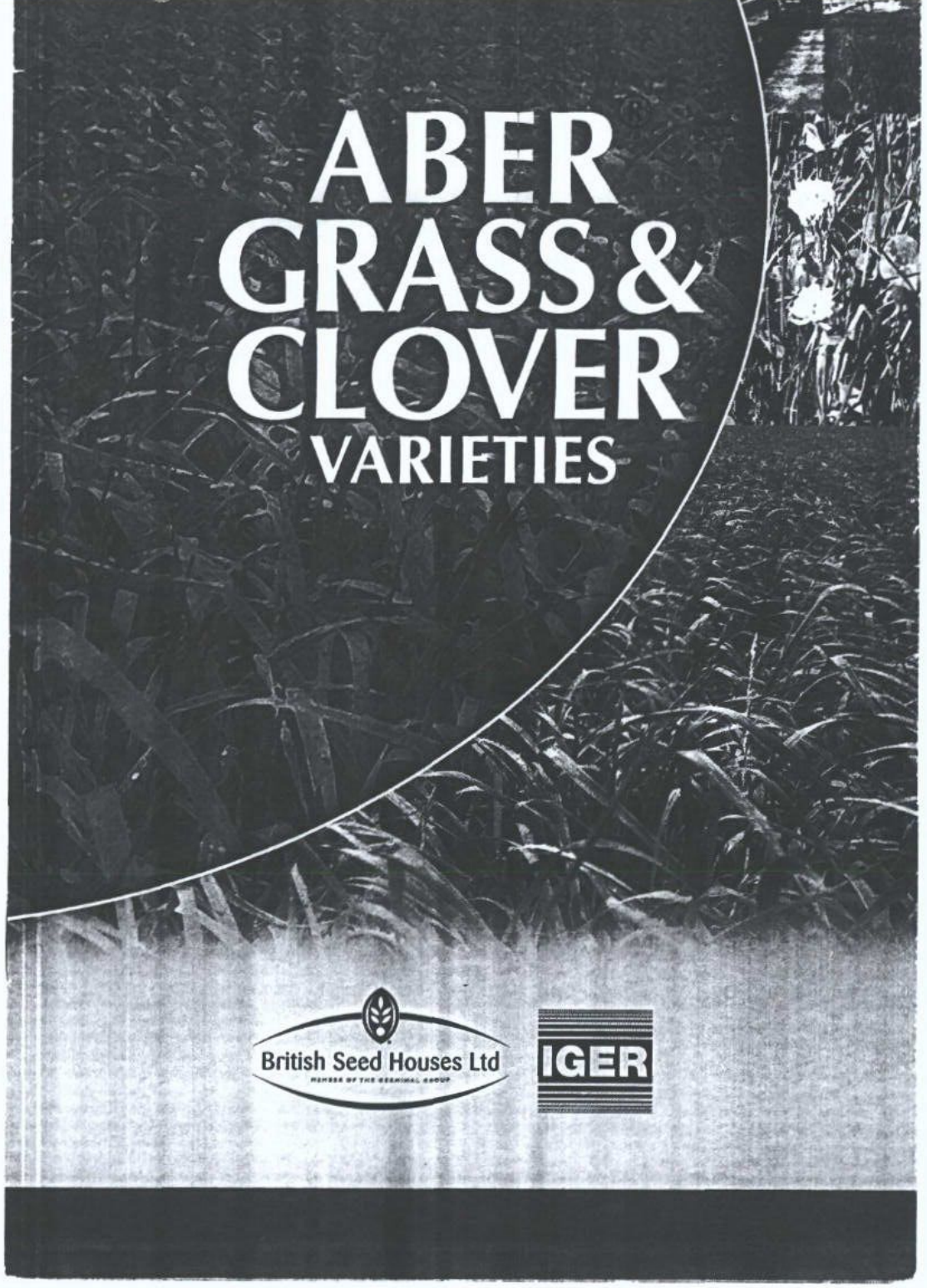
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Achieving Modern Grass Breeding Objectives

The emphasis of IGER's grass breeding programme changed in the 1980s with the realisation that the nutritional quality offered by new varieties would become as important as dry matter yield, as Britain's livestock farmers looked to continue improving the efficiency of production from homegrown forage. Environmental issues have also become more important and now have a far greater bearing on the direction of grassbreeding at IGER.

As a result characteristics such as sugar content and digestibility became primary objectives, alongside other important targets such as extending the grazing season and improving dry matter production per unit of nitrogen.

Twenty years later the emergence of the first AberHSG varieties offering significantly higher water soluble carbohydrate content and increases in milk and meat production potential as a consequence, is evidence that the change of emphasis was both correct and effective. These varieties also make more effective use of nitrogen, addressing a key environmental objective. The fact that these varieties have also kept pace with more traditional breeding objectives including dry matter yield, persistency, cold tolerance and disease resistance is testament to the overall success of the breeding programme and underlines the relevance of these developments to the farming industry.

Most encouraging of all is the knowledge that the improvements in nutritional quality seen so far are just the beginning. Whilst the intermediate diploid AberDart HSG, for example, has been recognised as the outstanding herbage variety of its generation, it is certainly not a one-off. There are now recommended AberHSG varieties in late diploid and hybrid ryegrass categories, and the next generation offering even greater potential is now emerging.

The ability to integrate grass breeding programmes with animal performance trials has long been a major strength of IGER and this has certainly been key in recent years, as improved nutritional value has become an objective. This integrated approach will remain vitally important as future objectives are fine-tuned, with parameters such as the consistency of protein content, the stability of desirable fatty acids (such as linoleic acid) and other quality traits coming into focus.

Dr Pete Wilkins
Manager of Grass Breeding, IGER

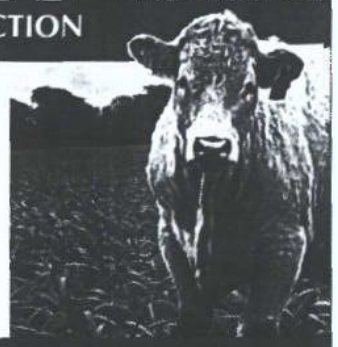
The Established Facts

There is now a bank of research evidence showing the significant animal performance benefits that High Sugar Grasses offer livestock farmers. Milk yield and meat production responses are a function of improved intakes and digestibility, plus the superior utilisation of grass protein that results from the higher readily available energy content of AberHSG grasses.

HIGH SUGAR GRASS FOR MILK PRODUCTION

- 6% more milk per cow over grazing season
- Dry matter intakes up by 2kg/head per day
- 3% improvement in diet digestibility
- 24% less feed nitrogen lost in urine

HIGH SUGAR GRASS FOR BEEF PRODUCTION



- Dry matter intakes increased by around 25%
- Higher forage intakes
- 20% higher daily liveweight gains
- Environmental benefit from reduced nitrogen pollution

HIGH SUGAR GRASS FOR LAMB PRODUCTION

- Higher forage intakes
- 20% higher liveweight gains
- 20% higher carrying capacity of HSG sward

Further details on these trials are contained in the booklet 'High Sugar Grass'.

Reference: SLP LK 0615

Strengthening the Case - Grazing

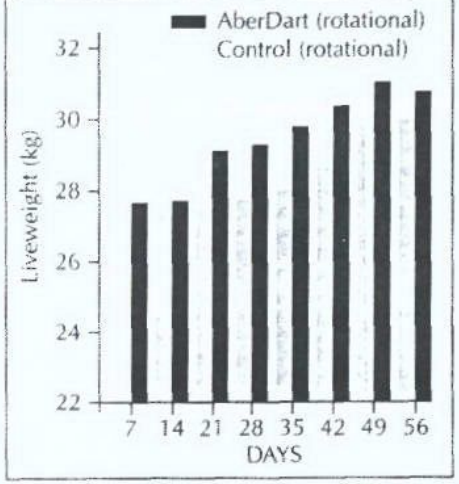
Data that strengthens the arguments in favour of the AberHSG phenomenon continues to emerge. The latest and arguably most comprehensive report yet comes from the jointly sponsored Sustainable Livestock Production, LINK programme, which features season long grazing trials conducted at IGER research farms using beef steers, lambs and dairy cattle.

Liveweight Gain (kg/d) in Beef Steers

Liveweight gain	AberDart	Control
First Year	1.12	0.95
Second Year	1.13	0.84

In dairy grazing trials the AberHSG variety AberDart produced very high mean milk yields, (31.7 kg/day of milk) from limited concentrate use (3 kg/day). The control variety in this trial showed similar levels of WSC and therefore produced a similar milk yield (31.6 kg/day). The control variety was the same in all three trials, but only showed this similar level of WSC in the dairy trial.

Liveweight Gain (kg) in Lambs



Strengthening the Case - Silage

Whilst the LINK study concentrates on the performance of AberHSG in a grazing context, other studies address the impact of High Sugar Grasses when ensiled. Recent research at IGER with beef cattle shows improvements in intakes and increases in the efficiency of rumen microbial protein synthesis when High Sugar Grass silage is fed.

Residual WSC in the HSG silage was 9.0% while that of the control variety was 5.5%. The higher WSC levels in the HSG silage led to the improved efficiency of feed N incorporation into microbial protein giving the potential to improve animal performance and reduce nitrogen excretion into the environment.

Reference: SLP LK 0638

Effects of Ensiled HSG

	HSG	Control
Intake		
Dry Matter (kg/d)	4.29	3.60
Organic Matter (kg/d)	3.95	3.33
Total Nitrogen (g/d)	93.1	91.3
Duodenal Flow		
Dry Matter (kg/d)	2.14	1.82
Organic Matter (kg/d)	1.67	1.34
Total Nitrogen (g/d)	96.2	74.9
Microbial Nitrogen (g/d)	63.2	41.7

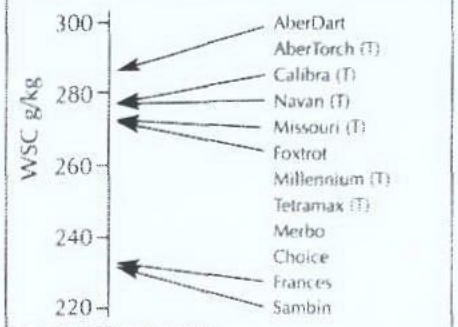
Source: Merry, IGER, 2003

AberHSG: Intermediate Perennial Ryegrass Varieties

AberDart HSG

AberDart HSG was the first AberHSG variety to appear on the NIAB, DARD and SAC recommended lists. In 2003 it became the first grass variety to win the prestigious NIAB Variety Cup for its outstanding improvement in quality. AberDart HSG is the highest yielding diploid ryegrass (NIAB 2003/4) with very good early spring growth, exceptional aftermath digestibility and Grazing D-Value, good ground cover and sound disease resistance.

Average sugar content of perennial ryegrass varieties under simulated grazing

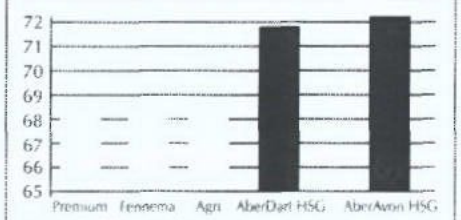


Source: Gilliland et al, 2002

A comprehensive review of trials conducted at IGER, DARD, DSV, Hunsballe and Peter Cates involving diploid and tetraploid perennial ryegrass varieties shows the superiority of AberDart HSG in terms of WSC content.

The significant improvement in AberHSG Grazing D-Values shows some correlation to the higher levels of WSC in these varieties under grazing management.

Grazing D-Value



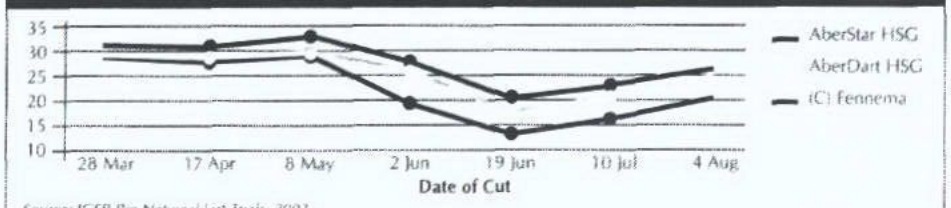
Source: NIAB R.L. 2003/04

THE NEXT GENERATION

AberStar HSG

AberStar HSG will be the next intermediate diploid to emerge from IGER's HSG breeding programme. A step forward on AberDart HSG for all the traditional characteristics, AberStar HSG offers another significant improvement in WSC content. Currently out-performing all other varieties in national list trials, AberStar will be considered for acceptance on to the recommended lists in 2005. In these trials it shows an increase in grazing dry matter of over 12% on existing varieties. Subject to national listing AberStar will be available in British Seed Houses mixtures from harvest 2004 onwards.

WSC Comparisons (%)



Source: IGER Pre-National List Trials, 2003

AberHSG: Late Perennial Ryegrass Varieties

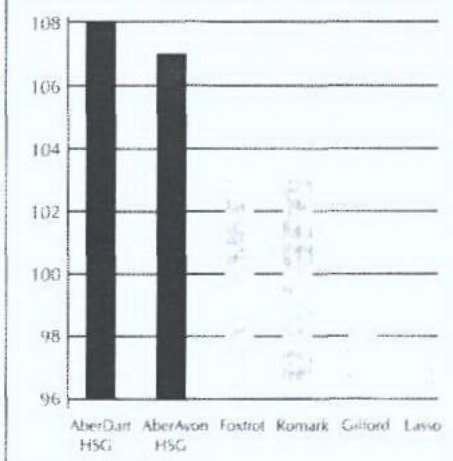
AberAvon HSG

AberAvon HSG is a high yielding late diploid offering a similar WSC content to AberDart HSG. It has the highest Grazing D-Value of all (72.23) perennial ryegrass varieties on the NIAB Recommended List 2003/4.

AberAvon HSG also excels in terms of dry matter yield. In the second harvest year in NIAB's simulated grazing trials, AberAvon HSG recorded a yield 7% higher than the control variety Fennema and is above all other late perennial ryegrass varieties on the recommended list.

AberAvon has shown an increased WSC content at every cut over a NIAB recommended tetraploid control variety.

Grazing DM Yield (% of control)



Source: NIAB R.L. 2003/04

WSC in AberAvon HSG and in Tetraploid Control Variety (g/kg)

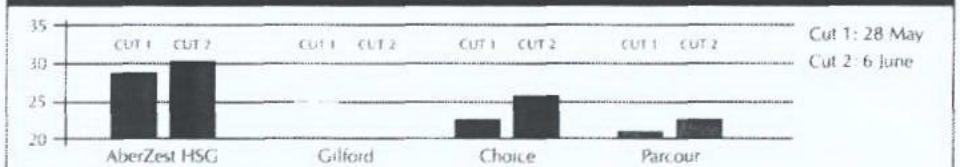
Variety	Cut 1 23 Mar	Cut 2 27 May	Cut 3 28 Jun	Cut 4 19 Jul	Cut 5 18 Aug	Cut 6 22 Sep	Cut 7 29 Oct	Mean
AberAvon HSG	112	242	299	315	236	210	234	236
Control (Tet)	111	235	275	287	214	188	220	219

Source: IGER Long Term Trial, 2002 Harvest Year

AberZest HSG

AberZest HSG is the latest late diploid to emerge from the IGER breeding programme. It is comparable to AberAvon HSG in terms of WSC content and offers superior ground cover to this variety. *Both AberAvon and AberZest are currently available.*

WSC (%)



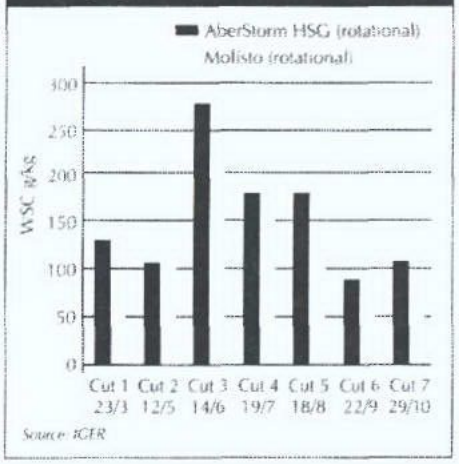
Source: IGER 1997 Sowing, 1998 Harvest Year

AberHSG: AberHybrid Ryegrass Varieties

AberStorm HSG

AberStorm HSG was the first AberHybrid HSG variety. Earlier heading than the successful intermediate Hybrids AberLinnet and AberExcel, AberStorm HSG is high yielding for grazing and conservation and provides outstanding early spring growth at 148% of the control.

WSC (g/kg) of AberStorm and Molisto

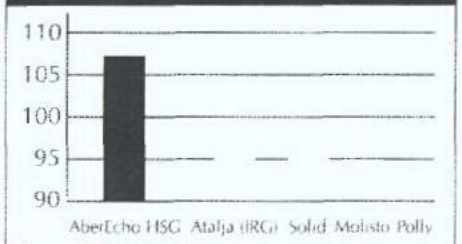


Source: IGER

THE NEXT GENERATION

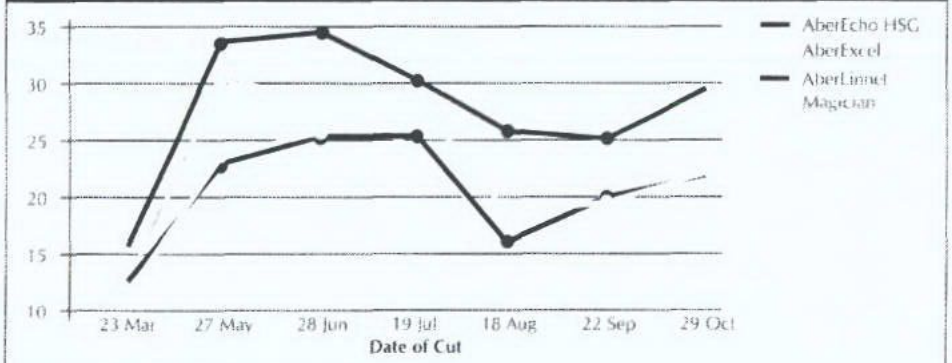
AberEcho HSG is the latest AberHybrid HSG variety from the IGER breeding programme. It is the highest yielding Hybrid Ryegrass variety available, out-yielding all the Hybrids and even the Italian ryegrasses listed by NIAB (2003/4) in the first harvest year. It has all the advantages of the AberHybrids and will persist in rotational grazed and cut leys for up to five years (twice as long as Italian ryegrass). AberEcho HSG also shows excellent disease resistance and mid season quality.

Mean DM Yield%



Source: NIAB RL 2003/04

WSC Comparisons (%)



Source: IGER Long Term Trial, 2002 Harvest Year

Maximising the Aber Advantage

Great progress is being made by IGER's grass breeding programme in terms of the nutritional quality of new ryegrass varieties. The breakthrough has come with the emergence of perennial ryegrasses such as AberDart HSG, AberAvon HSG, and AberStorm HSG. Already the next generation is showing a further significant step on, with varieties like AberStar HSG and AberEcho HSG Hybrid Ryegrass coming to the fore. These varieties show a significant increase over AberDart. The improvements are set to continue, as over twenty years of focused breeding comes to fruition.

The potential benefits for livestock farmers from these improved grass varieties are real and highly significant. Tangible performance increases in terms of milk yield and liveweight gain have been demonstrated in trials, and forward-looking farmers are already seeing positive results for themselves.

GETTING THE MIXTURES

In order for the farmer to gain full advantage it is important not to dilute the potential. This means using mixtures that are made up entirely of the appropriate AberHSG varieties and are not compromised in any way with lesser conventional inclusions. This is now possible, due to the range of superior varieties coming through the IGER breeding programme.

Dilution of mixtures with non AberHSG varieties will certainly reduce the benefits. Animal studies on AberHSG varieties at IGER have in general shown that in order to generate a significant animal performance benefit, a grazing sward must provide a minimum increase in the level of water soluble carbohydrate of four percentage points. However, for silage production bigger differences may be required in the standing crop.

Hence, a farmer growing Mixture B instead of Mixture A may not reap any tangible animal performance benefits, despite using a mixture with an AberHSG component.

Mean WSC Content (%)

HSG Mixture (14kg)	WSC (%)	AberDart + Non HSG (14kg)	WSC (%)
3kg AberAvon (Late PRG Dip)	24.90	3kg Control (Int PRG Dip)	21.24
5kg AberEcho (Hybrid Tet)	27.90	3kg Control (Late PRG Tet)	21.87
3kg AberStar (Int PRG Dip)	24.90	5kg AberLinnet (Hybrid Tet)	20.71
3kg AberDart (Int PRG Dip)	23.28	3kg AberDart (Int PRG Dip)	23.28
Average WSC (%)	**25.63**	**Average WSC (%)**	**21.62**

WSC data based on IGER Long Term Trial, 2001 sowing, 2002 harvest

This extrapolation of data from variety trials shows the importance of maintaining a full complement of AberHSG varieties in a mixture.

Achieving Modern Clover Breeding Objectives

The emergence of new varieties offering higher yields, greater versatility of use and – perhaps most importantly – increased reliability is changing traditional perceptions and underpinning a significant new role for white clover in sustainable livestock production.

No longer is white clover associated solely with low input / low output and/or organic systems, but is increasingly central to the most progressive forage based enterprises. As a companion to modern grasses in swards for either grazing or conservation, it is a species that addresses many of the modern farmer's priorities – from the cost effective provision of homegrown protein to reduced reliance on fertiliser nitrogen.

Eighty years of clover breeding at IGER have helped drive this revolution, and – as new objectives ensure continuing progress – this programme is set to maintain an essential role in future development.

Reliability has been an Achilles heel for white clover in decades past, but optimum targets of a 30-35% contribution to total sward dry matter are now being achieved under a variety of management systems – and with unprecedented levels of consistency. This is due in part to IGER being focused on key breeding objectives, such as winter hardiness, pest/disease resistance and nitrogen tolerance.

Working closely with modern grass breeding is vital for the clover breeder to ensure a level of compatibility that will allow optimum sward composition. This is a major strength of the IGER programme and a key factor in the emergence of white clover in modern livestock production.

A close affinity with agricultural practice remains a strong feature of IGER's work. Selection and testing takes into account performance in the silo and in the rumen, as well as in the field under simulated farm conditions. This is only possible because of the strong links with IGER's animal scientists and such cooperation will become all the more important as breeding objectives become more focused on improving feed quality and reducing environmental impacts.

Michael T Abberton

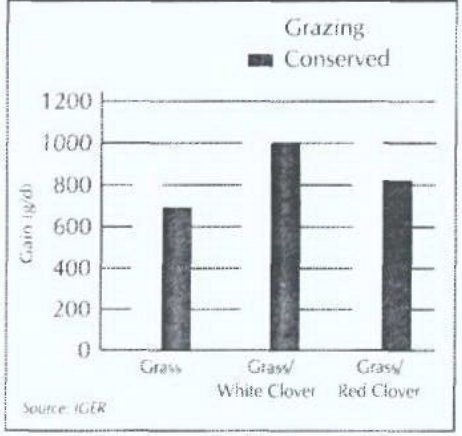
Dr Michael Abberton
Head of Clover Breeding, IGER

Forging an Effective Combination

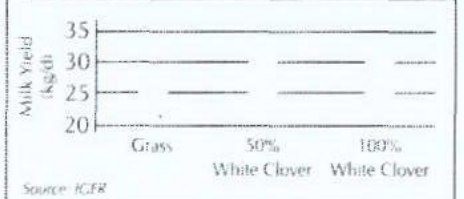
The benefits of combining grass and white clover in swards for both grazing and conservation are long established and well proven. The most important benefits can be summarised as:

- improved forage quality and feed value due to a boost in digestibility, intake potential, and protein and mineral content of the sward
- reduced reliance on fertiliser due to nitrogen fixation
- "soil structuring" by white clover root systems that can help to overcome problems of soil compaction

Liveweight Gains on Clover



Milk Yield



Accumulated experience and scientific evidence indicate that the optimum balance is achieved with a clover content of 30-35% of the total annual dry matter yield of the sward.

In reality, the clover content of a mixed sward will vary from a low level in the spring (as low as 5% of total dry matter) to as high as 60% in July/August. This level of variability in the clover content is not ideal from the perspective of managing the feeding value for livestock and can also cause deterioration in sward quality over time. Hence, greater compatibility between grass and white clover varieties is vital in order for livestock farmers to gain maximum benefit.

Breeding for compatibility

A compatible grass/clover mixture is one with a clover content that is sufficiently large to optimize the nutritional and nitrogen fixing attributes of the clover when growing with a high yielding companion grass.

Grass and clover varieties differ in their aggressiveness towards each other due to their abilities to compete for nutrients, water and light. At IGER, breeding for general compatibility is high on the agenda and varieties are routinely tested for this attribute.

Evidence of the progress that takes clover into a new era of utilisation is summarised by three key areas:

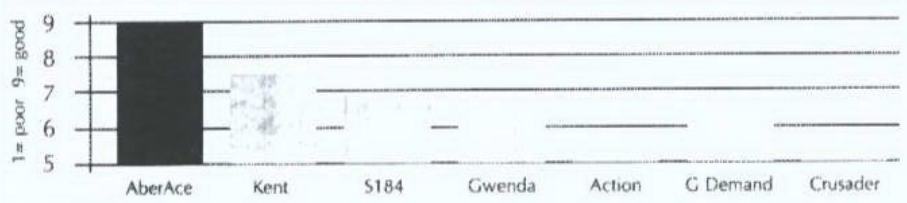
- Annual clover contributions of 30% or greater from IGER-bred varieties have been proven experimentally and are now being seen on the farm
- Compatibility with modern Italian, AberHybrid and high yielding perennial ryegrass varieties
- Evidence from long-term experiments showing effective levels of clover being maintained in swards for many years under high and low N regimes

AberAce
This new variety has the smallest leaf size of any on the recommended lists and was bred specifically for continuous sheep grazing in lowland and upland conditions. It has an exceptionally dense network of stolons and will persist well under the most rigorous of sheep grazing systems.

AberCrest
Productive under sheep grazing as well as under rotational grazing by dairy cows. Bred from wild populations collected in Switzerland, AberCrest's major attribute is its good cold hardiness and stolon survival. It possesses a thick stolon compared with other small leaved varieties and shows more rapid regrowth following cutting or grazing in spring.

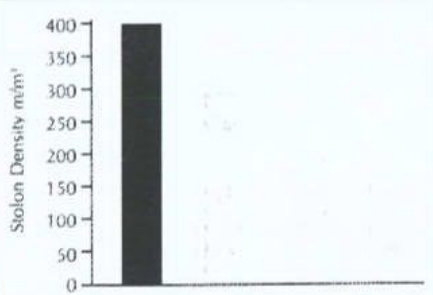
AberPearl
High yielding small leaved variety. Bred from a gene pool of 8 small and medium leaf size varieties showing early recovery after winter (greenness of stolon and leaf). Extremely persistent under rotational or continuous sheep grazing. Excellent ground cover.

Autumn Ground Cover Rating

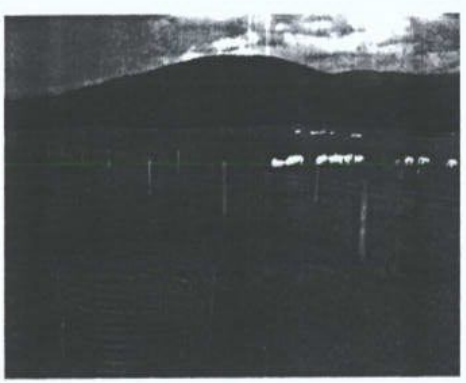


Source: NIAB R.L. 2003/04

AberAce Stolon Density



Source: IGER, Bronydd Mawr



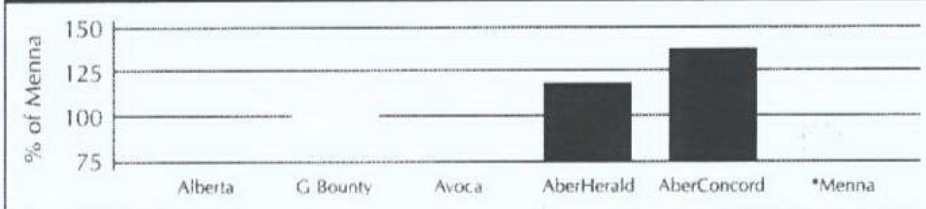
White clover varieties being assessed for persistence by sheep at IGER

AberHerald
With a relatively small leaf size, this variety is high yielding and has excellent winter survival combined with the ability to regrow rapidly in spring. AberHerald is also tolerant of nitrogen, and has been maintained in swards at 25% with inputs of 380kgN/ha. This variety is suitable for rotational sheep and cattle grazing systems, and can also be cut for silage.

AberDai
This variety was bred from winter hardy material to provide flexibility in response to various management regimes. It offers high yields and survives well in systems ranging from continuous sheep grazing to rotational grazing by cattle or sheep.

AberConcord
A new medium leaved variety producing yields as great as some large leaved clovers and gives good performance over a wide range of N inputs (0-450kg/ha). This trait contributes to a stable clover yield over several years by allowing clover to tolerate the build-up of nitrogen in the soil. AberConcord also provides good winter survival and spring growth, regrowing rapidly following cutting and grazing, and is suitable for a range of management systems, from rotational grazing by sheep and cattle to conservation.

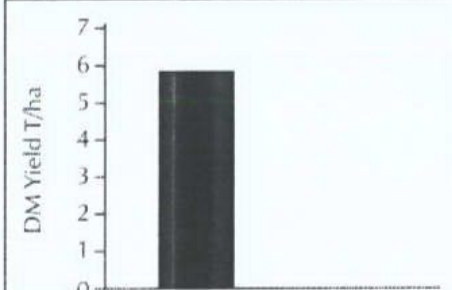
Relative DM Yield (3rd Harvest Year)



Source: NIAB R.L. 2003/04

(*Yield of Menna = 2.93 t/ha)

Annual DM Yield (150kg/ha/N/yr)



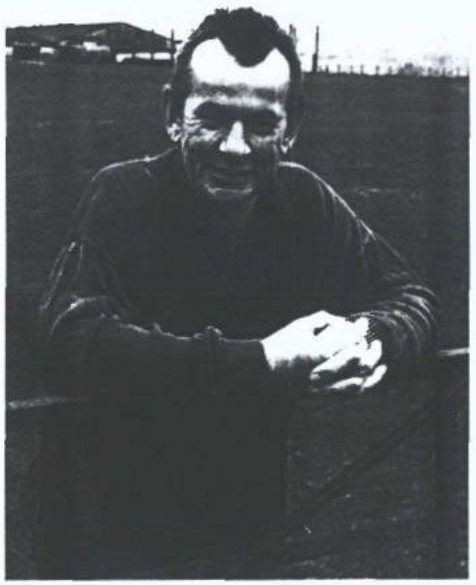
Source: IGER

Response to Grazing Management

	Annual Dry Matter Yield (t/ha/annum)	
	Continuous sheep grazing	Cutting
Olwen (large)	2.0	7.5
Menna (medium)	3.7	5.1
AberDai (medium)	4.1	6.9

Source: IGER

Yield Boost For High Performing Holsteins



Jim Reid was one of the first producers in Scotland to sow a significant acreage of AberHSG and now has around 120 acres of leys that contain AberDart and AberAvon at Bengal Farm near Lockerbie.

Grass reseeds on the 750-acre mixed farm are expected to last 5 years and must be capable of top performance under cutting and grazing to match the demands of the high yielding dairy herd and followers. A flock of 500 ewes and their lambs also graze the farm's pasture.

The first AberHSG leys were sown under barley in 2000, but it was in the aftermath of foot and mouth in 2001 that a major reseeding programme took place creating the opportunity for widespread improvement of pastures.

The sowing of AberHSG leys coincided with the arrival of a new herd of dairy cows – so direct comparisons are difficult - but cows have certainly performed very well, sustaining a current average yield of 9,200 litres/cow. Jim Reid reckons that an improvement of around 500 litres per animal can be attributed to the better quality of the new leys over the past two seasons, which represents a yield boost of 5.7% and is very close to the 6% figure recorded by IGER in dairy trials.

"The sheep also find the new pastures very palatable," he says. "Ewes and lambs graze more vigorously. There is lower wastage and the high sugar grass leys require less topping. Most of the homebred lambs finished off grass last season, but it's difficult to compare the old and new leys precisely because lambing was around two months later than normal in 2002, due to readjustments following restocking.

"Silage quality from the new leys is well above average. Dry matter yields have been good and we hope that the higher sugar levels of the new varieties will continue to play a part in producing silage of consistently better quality to complement the high starch, fermented whole-crop cereals that we also conserve for winter rations."

AberHSG Gives Ram Rearer Added Muscle

High Sugar Grass is boosting performance on a sheep farm in Worcestershire, while reducing reliance on bought-in feed.

Since planting his first 62 acres of AberHSG varieties Geoff Probert has cut back concentrate feeding to his pedigree rams significantly, yet still turns out Charollais and Texel tups in top condition to average well in excess of £425 this year.

Farming 480 acres at Northingtown Farm, Holt Heath near Worcester Geoff Probert runs 300 pedigree Texel and Charollais ewes plus 1200 Mule cross Texel commercial ewes and aims to maximise production from home-grown grass and fodder crops.

"We are producing breeding rams to sell to the best commercial sheep producers and we want to have customers returning to us year after year for more," said Geoff.

"Commercial sheep farmers need their tups to last so it's no good feeding a pedigree tup with expensive coarse mix for them to get on to a customer's farm and just melt."

The first 62 acres of AberHSG mixtures were planted in September 2002 and Geoff was impressed by their vigour. The leys produced an early spring flush of grass and then continued throughout the summer, recovering well after the drought on the sharp loam land at Holt Heath. A contented flock at grass was one of the first signs that convinced Geoff of the value of his AberHSG investment.

The mixture includes the AberHSG varieties AberDart, AberEcho, AberAvon and AberStorm, with clover being oversown at a later stage.

The spring flush of quality grass helps to finish 1000 or so spring lambs from the mid-January lambing commercial flock and then, to boost the grass lull in a dry July and August, kale provides a quality fresh bite for the stock. After that turnips and swedes come into their own, and then it's back round to spring grass again.

"You have to be prepared to take on new ideas," concludes Geoff. "I'd followed the research on High Sugar Grass at IGER and realised it was a way of producing quality rams without the costs getting out of hand."