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STOCKER PERFORMANCE OF ST. CROIX, DORSET, AND CROSSBRED LAMBS ON WHEAT PASTURE. Jeremy D. Knox, Michael A. Brown, and William A. Phillips. Redlands Community College, El Reno, Oklahoma 73036 and USDA-ARS, El Reno, OK 73036.

Four breed groups of lambs (Dorset x Dorset, DxS; St. Croix x St. Croix, SxS; Dorset x St. Croix, DxS; St. Croix x Dorset, SxD; $n=61$) were used to evaluate adaptation to wheat pasture. After weaning, one-half of each breed group was placed in drylot (DL) or on wheat pasture (WP). Lambs in DL were fed a ration balanced to match gains of WP lambs. Weight gains in DL and WP lambs were measured for two three-week periods, after which all lambs grazed wheat pasture. Gains were then measured for four weeks to observe wheat pasture adaptation of DL lambs. Wheat pasture lambs gained less ($P<.05$) than DL lambs for weeks 1-3 (Period 1), but DL and WP lambs were similar for weeks 4-6. When all lambs were moved to wheat pasture (week 7-10, Period 3), gain of DL lambs was less than WP lambs ($P<.01$). Using differences between DL and WP as a measure of magnitude of adaptation, DxS and SxS appeared to adapt more readily than SxD and DxS in Period 1. The same trend occurred in Period 3 in the same breed groups. However, adaptation time was similar among all breed groups in Periods 1 and 3. We conclude that adaptation to wheat forage is necessary and that genetic differences in magnitude but not time of adaptation exist.

DUAL-PURPOSE AND GRAIN-ONLY MANAGEMENT SYSTEM EFFECTS ON SHOOT AND N USE TRAITS OF WHEAT AT ANTHESIS. Vernon A. Clifford¹, Charles T. MacKown², Brett F. Carver³, and Eugene G. Krenzer, Jr.³. ¹Redlands Community College, El Reno, OK 73036; ²USDA-ARS, Grazinglands Research Laboratory, El Reno, OK 73036; ³Department of Soil and Plant Science, Oklahoma State University, Stillwater, OK 74078.

Growing winter wheat (*Triticum aestivum* L.) as a dual-purpose crop for both forage and grain is an important management tool for many producers in the Southern Great Plains that offers economic advantages not enjoyed by producers relying on wheat solely for forage or a grain crop. While much more is known about management effects of dual-purpose wheat on grain yield, documentation of the impacts of grazing on many physiological traits of wheat that could affect yield and grain quality are unknown. Experiments conducted at the Oklahoma State University Wheat Pasture Center near Marshall (1998-2000) compared anthesis shoot and N use responses of wheat grown either as a dual-purpose crop or for grain only. A range of wheat cultivars that varied in plant height and yield potential was used. Averaged across wheat cultivars, flag leaf weights from the dual-purpose system were 24 to 31% less with N concentrations up to 15% less than in flag leaves from the grain only system. In one of two years, shoots collected from 1 m of row of dual-purpose wheat weighed 24% less than that of the grain only system and accumulated 28% less N. These traits were not significantly different ($P\leq 0.05$) in the second year. In year 2000, single culms of dual-purpose wheat at anthesis had spikes with 25% less dry weight and 5.9% lower N concentration and a total culm dry weight 9.3% less and N content 23% less than culms of grain-only wheat. These results demonstrate that several traits of wheat that are linked to grain filling and N accumulation can be adversely affected by the dual-purpose management system, but the magnitude and occurrence of these effects depends on environmental factors.

THE GAME OF LIFE HISTORY. Erica A. Corbett. Department of Biological Sciences, Southeastern Oklahoma State University, Durant, OK 74701.

Simple simulation exercises can enhance student learning and enjoyment in ecology classes. The concept of allocation patterns and trade-offs is well suited to simulations. I developed a "game" to reinforce students' understanding of the trade-offs involved in semelparity vs. iteroparity, reproductive effort, predator avoidance, and overcoming prey defenses. Students observed which patterns of allocation were optimal for their particular species. Moreover, students were exposed to how environment, specifically disturbance frequency, affects what life-history patterns succeed. Students were given counters or tokens that represent energy and resources to be allocated. Each student represented a species – producer, herbivore, or carnivore. Six species were then grouped to form an "ecosystem" – three producers, two herbivores, and one carnivore. Each species' use of energy included reproductive strategies, survival, and defense. The game included "feeding rounds" where predators take energy from their prey. Each ecosystem was also subjected to a different disturbance regime (frequent, moderate, low, or no disturbance). Students whose species "died out" (either were totally consumed by a predator or failed to reproduce successfully) were given the option to start over with a different allocation pattern. Student response and learning were measured using pre- and post-tests and by evaluating responses to questions about allocation patterns after the activity.

A COMPARISON OF ANIMAL PERFORMANCE UNDER EITHER CONVENTIONAL CONFINEMENT FEEDING OR FEEDING ON PASTURE FOR FINISHING BEEF CATTLE. Wes L. Graham, William A. Phillips and Michael M. Brown. Redlands Community College, El Reno, OK 73037 and USDA-ARS Grazinglands Research Laboratory, El Reno, OK 73036.

Oklahoma is the production center of the U.S. beef cattle stocker industry. With improved cattle genetics and stocker management, stockers are heavier at the end of the production cycle and are sometimes discounted when sold in the spring. Stocker producers need management options that allow them to retain ownership of heavy stockers and finish them with on-farm resources to improve net returns. Our objective was to evaluate a new on-farm finishing system. Stockers (n=224) were assigned to be finished under a conventional confinement feeding systems (feedlot) or on pasture with ad libitum access to a high grain diet (pasture). Stockers finished on pasture consumed 220 kg less feed than stockers finished in the feedlot (1087 vs 1311 kg). An added bonus in the pasture system was the distribution of the animal waste generated over the pasture by the stocker, which reduced the cost of waste disposal associated with the feedlot system. We conclude that heavy stockers can be finished on grass pastures using available on-farm resources with less feed inputs as compared to a conventional feedlot feeding system and that the disposal of animal waste is done at less cost under the pasture system.

ISOLATION AND IDENTIFICATION OF PATHOGENIC AMEBAE FROM TAYLOR FERRY, FORT GIBSON LAKE, OKLAHOMA. Kristopher K. Hart, Keenan L. Ferguson, Marsha J. Howard, and David T. John. Department of Biochemistry and Microbiology, College of Osteopathic Medicine, Oklahoma State University, Tulsa, OK 74107.

Pathogenic free-living amoebae are able to cause serious disease in humans, including a fatal brain infection and a serious eye infection. The first confirmed case of primary amoebic meningoencephalitis (PAM), caused by *Naegleria fowleri*, in Oklahoma occurred in August

1998 when a 3-year-old girl became infected while playing in the water at the edge of Taylor Ferry, Fort Gibson Lake. An autopsy confirmed the diagnosis of PAM by histologic identification of amebae in brain tissue and by cultivation of amebae in axenic medium. The isolate was designated as HBT-1. During the summer of 1999, we sampled the water of the swimming area of Taylor Ferry and obtained 16 pathogenic ameba isolates. Ten of the isolates have been tentatively identified as *N. fowleri*, 4 as *N. australiensis* and 2 as *Acanthamoeba* sp. Fifteen of the isolates were recovered during months of exceptionally heavy rainfall, suggesting that perhaps rainfall may influence the presence of these amebae in the environment. Heavy rainfall may agitate the water sufficiently to suspend greater numbers of amebae in the water. Alternatively, excessive rains may have washed greater amounts of coliform bacteria from the surrounding farms into the water, thus providing a greater food supply and therefore more amebae.

ANALYSIS OF CARBON DIOXIDE, VINYL CHLORIDE, AND METHYL t-BUTYL ETHER (MTBE) IN AQUEOUS SOLUTION USING THE BUBBLE STRIPPING METHOD. D.H. Kampbell¹, B.C. Roehl², and D.M. McInnes². ¹US-EPA, Office of Research and Development, National Risk Management Laboratory, Subsurface Protection and Remediation Division, Ada, OK 74820; and ²East Central University, Ada, OK 74820.

The bubble stripping procedure is applied at field sites by filling a sample cell with well water, and then charging it with 20 mL of air to produce a head space over the water. The head space is the "bubble" into which any gases in solution will partition. A peristaltic pump is used to pump the well water through the cell such that a stream of water flows through the head space and produces agitation in the aqueous phase. Pumping is continued until equilibrium is established between the aqueous phase and the head space. Gas chromatographic analysis is employed to determine gaseous concentration in the head space, which allows concentration in solution to be calculated using Henry's law. The Bubble Stripping Method was applied in the laboratory to determine carbon dioxide, vinyl chloride, and MTBE concentrations in aqueous solution. A solution flow rate of 300 mL/minute through the cell was optimal. Employing a specially designed apparatus, it was concluded that the stripping process was complete within ten minutes.

THE EFFECT OF POINT SOURCE EFFLUENT ON HYPORHEIC COMMUNITIES IN FOUR OZARK STREAMS. G.W. Hunt. Division of Science and Math, Tulsa Community College, Tulsa, OK 74119

The impact of treated sewage on the hyporheos was investigated at 4 Ozark streams (Osage, Sager, and Spring creeks and Columbia Hollow) in Benton County in northwestern Arkansas. Samples were collected on 3 dates during the period June 9-August 11, 1997, at 4 stations on each stream. One station was located above the discharge and 3 stations were located downstream of the discharge at various distances. Samples were collected using the Bou-Rouch method at well depths of 5–20, 30–45, 60–75 and 100–115 cm below the stream bottom. The fauna in Columbia Hollow was dominated by oligochaetes and nematodes, whereas harpacticoid and cyclopoid copepods and ostracods were the dominant taxa in the other streams. Invertebrate densities and taxon richness above and below the discharge points did not differ significantly. Ordination and Spearman rank correlations indicated that community composition in the four streams is strongly dependent on DO availability, which decreased with depth in the hyporheic zone. Low DO levels in the hyporheic zone suggest that organic loading from nonpoint source runoff may be producing adverse impacts not previously indicated during monitoring of benthic macroinvertebrates.

PRODUCTION OF BaCMV pp65 and pp71 RECOMBINANT GST FUSION PROTEINS FOR DIAGNOSTIC ELISA. R. Preston Rogers and Earl L. Blewett. Department of Biochemistry and Microbiology, College of Osteopathic Medicine, Oklahoma State University, Tulsa, OK, 74107.

As the demand for organs needed for transplantation in humans increases, other viable xenogeneic donor sources, such as baboons, have become attractive candidates. Baboon cytomegalovirus (BaCMV) found in organs for transplantation can pose serious risks to the immunosuppressed recipients. Successfully screening baboon populations for antibodies to BaCMV requires a sensitive diagnostic ELISA to identify infected individuals. Our current assay uses BaCMV-infected cell proteins that are expensive to produce and contain infectious virus. Production of recombinant viral proteins in bacteria will provide both a safe and economical alternative for the diagnostic ELISA. The phosphoprotein 65 and 71 genes (pp65 and pp71) found in cytomegaloviruses code for highly immunogenic tegument proteins. We have cloned and expressed these genes in bacteria as glutathione-S-transferase (GST) fusion proteins and highly purified them for use in ELISA screening of sera from BaCMV-infected baboons. The data demonstrate that some of the sera tested reacted strongly with the recombinant antigens while others did not. Production of additional recombinant antigens is needed for more definitive screening of sera from infected baboons.

USING A LAMBDA EXPRESSION LIBRARY TO IDENTIFY IMMUNOLOGICALLY IMPORTANT BABOON CYTOMEGALOVIRUS ANTIGENS. Tami G. Ross and Earl L. Blewett. Department of Biochemistry and Microbiology, College of Osteopathic Medicine, Oklahoma State University Tulsa, OK 74107.

The use of non-human primates as organ donors raises the possibility for zoonotic diseases resulting from infectious agents present in the donor tissue. Cytomegalovirus is the most commonly transmitted virus in human transplantation procedures and a major cause of many problems in immunosuppressed transplant recipients. Development of a sensitive and reliable diagnostic assay for the detection of baboon cytomegalovirus (BaCMV) in donor baboons and in humans receiving baboon xenograft transplants is our goal. We have constructed several λ TriplEx libraries using randomly sheared genomic DNA and screened them with BaCMV-positive sera to identify λ virus clones expressing useful immunogenic proteins. One important protein we have identified using this system is the BaCMV homologue of the human CMV pp65 protein. This protein and other promising diagnostic antigens have been expressed in a bacterial expression system. These strong antigens will be used to develop more sensitive diagnostic ELISAs and western blot assays allowing detection of infected baboons and humans.

INTRATHYMIC TRANSPLANTATION OF GENETICALLY MODIFIED THYMIC EPITHELIAL CELLS. S.S. Sands and R.J. Ketchum. Department of Anatomy & Cell Biology, College of Osteopathic Medicine, Oklahoma State University, Tulsa, OK 74107.

Thymic epithelial cells (TEC) are involved in positive clonal selection and may be involved in negative selection in immune education. The isolation, genetic modification, and retransplantation of TEC may allow reeducation of the host immune system. We investigated the isolation, culture, and in vitro manipulation of rat TEC. Wistar-Furth rats (4-5 days old; N=6) were sacrificed and thymi removed. TEC were isolated by mincing and collagenase digestion, and plated in prolactin and IGF-1 supplemented media. Immuno-

cytochemistry and transmission electron microscopy (TEM) were used to verify the epithelial origin of these thymically derived cells. Indirect Ab labeling revealed intracellular cytokeratin. Desmosomes and keratin intermediate filaments, typical of epithelial cells, were visualized by TEM. Green fluorescent molecular probes (CMFDA, 10 nM) were used to label TEC. Cells were collected on a Millipore PTFE filter, fixed, embedded, and sectioned. Anti-fluorescein mAb/DAB staining demonstrated positivity for CMFDA. Labeled TEC were injected intrathymically (IT) via indirect visualization. Two days later, recipients were sacrificed and thymi excised, fixed in Bouin's solution, embedded, and sectioned. Immunocytochemical staining with anti-fluorescein mAb demonstrated some positivity within the injection tract in the thymus. In vitro transfection of TEC with the red fluorescent protein (RFP) reporter gene resulted in TEC expression of RFP. Continuing studies will determine whether IT transplantation of genetically modified TEC can alter host immunity without affecting appropriate immune responsiveness. (OSU-COM intramural support).

EFFECT OF HIV-ASSOCIATED PROTEINS ON DOPAMINERGIC NEURONS. D.R. Wallace. Department of Pharmacology & Physiology, College of Osteopathic Medicine, Oklahoma State University, Tulsa, OK 74107.

HIV-associated proteins, gp120 and Tat, contribute to neurotoxicity, dementia and Parkinson-like symptoms coinciding with late-stage AIDS. Yet, the effects of gp120 and Tat on dopaminergic neurons are unclear. Male rats (6-months old; N=4) were sacrificed and striata were removed on ice. Crude synaptosomes were obtained as previously described. Pargyline (100µM) was added to prevent oxidation by monoamine oxidase. Nonspecific uptake was determined by 1 µM mazindol. Synaptosomes were incubated for 15 minutes at 22°C in the presence of [³H] dopamine, and either buffer (control), or varying concentrations of gp120 or Tat. Uptake was terminated by filtration and washing with ice-cold Tris-HCl buffer. To examine the effect of gp120 and Tat on the DAT, synaptosomes were incubated in the presence of either 0 (control), 30 pM gp120 and 9 concentrations of [³H] dopamine (10-2000nM). Both gp120 (30pM) and Tat (60nM) inhibited [³H] dopamine uptake. Inhibition of uptake was likely due to an increase (73%) in Km of the transporter for [³H] dopamine. Reduction in transporter affinity could be due to damage of the transporter protein by the oxidative stress following gp120 or Tat administration as indicated by increased carbonyl formation. (Supported by OSU-COM intramural funds & NIH DA13137)

GENERATION OF LARGE NESTED DELETIONS IN A 200 KB MOUSE GENOMIC BAC CLONE. Todd E. Bridges and Jonathon S. Coren, Department of Biology, Southwestern Oklahoma State University, Weatherford, OK 73096.

Genomic libraries are being used by researchers as the substrates for determining the sequence of a variety of organisms. Generating nested deletions in individual library members is useful in physical and functional mapping of one boundary of a gene or a regulatory element and in aligning the sequence of clones containing highly repetitive DNA sequences. In this study, large nested deletions were generated by retrofitting a 200 Kb mouse genome BAC clone (b637) with random insertions of the pTnBAC//loxP transposon cassette, which contains a loxP site and the chloramphenicol resistance marker. P1 *vir* infection was used to generate deletions in a single clone and to transduce them into new cells. BAC plasmid DNA was isolated from several transductants, and then the DNA's were digested with NotI or BamHI and analyzed by agarose gel electrophoresis. Many clones showed evidence of being nested deletions although not all deletions were related to one another. Also, certain deletion events were preferred since they were recovered at a much higher frequency. In an

attempt to randomize the deletion events, one hundred b637-pTnBAC//oxP transformants were pooled, and then deletions were generated by the same method. This modified procedure did increase the randomness of the insertions. The fact that we recovered a series of independently nested deletions could be due to prior rearrangements and/or deletion events occurring between the many repetitive elements present in this BAC clone before the transposon-induced events occurred.

UREA TRANSPORT IN THE INNER MEDULLARY COLLECTING DUCT (IMCD): POSSIBILITY OF AN IMIDAZOLINE MEDIATED MECHANISM. A. Rouch and L. Kudo. College of Osteopathic Medicine, Oklahoma State University, Tulsa, OK 74107 and Faculty of Medicine, University of Sao Paulo, Sao Paulo, Brazil.

Urea transport in the IMCD is important for generating a concentrated urine. Arginine vasopressin (AVP) stimulates urea transport. The purpose of this study was to test imidazoline selective agents on AVP-stimulated urea transport in the rat IMCD. These agents are dexmedetomidine, clonidine, and oxymetazoline which are also selective for alpha-2 adrenoceptors. We measured urea permeability (Pu) in the isolated Wistar rat IMCD. Three separate protocols were conducted (i.e., one for each agent) and n=5 for each protocol. Pu was increased with 220 pM AVP followed by adding the agent at 10nM, 50nM, 100nM, and 1M. Dexmedetomidine failed to affect AVP-stimulated Pu whereas clonidine and oxymetazoline significantly inhibited Pu with ED50 of 281.7nM and 168.9nM, respectively. In previous studies we showed that all three agents significantly inhibited AVP-stimulated water permeability. To begin testing the possibility that inhibition of AVP-stimulated Pu occurs via an imidazoline mechanism we tested the imidazoline selective antagonist BU239 on Pu. BU239 at 100nM reversed inhibition of AVP-stimulated Pu whereas it did not reverse water permeability. The evidence suggests that imidazoline receptors play a role in modulating urea transport.

STRUCTURAL AND PHYSIOLOGICAL CHANGES IN STAPHYLOCOCCUS AUREUS STEP-WISE ADAPTED TO VANCOMYCIN RESISTANCE. Marvita D. McGuire and Robert S. Conrad. Department of Biochemistry & Microbiology, College of Osteopathic Medicine, Oklahoma State University, Tulsa, OK 74107.

Vancomycin is a glycopeptide that disrupts bacterial cell wall synthesis by binding to the D-alanine-D-alanine dipeptide of the peptidoglycan. Vancomycin is the drug of choice for methicillin-resistant *Staphylococcus aureus* (MRSA). Prolonged *in vitro* or *in vivo* exposure to vancomycin contributes to decreased susceptibility of *Staphylococcus* to the presence of the antimicrobial. Structural changes in the adapted cell include thickened cell wall, multiple septa and amorphous material binding to the periphery of the cell. Polymerase Chain Reaction (PCR) found that not only was the penicillin binding protein 4 (PBP4) gene absent in the adapted strain, but also the *mecA* gene. The adapted strain, but not the parental strain, demonstrates resistance to lysostaphin suggesting alterations in the pentaglycine cross-linkage. The adapted strain had an increased generation time. Physiological changes in the adapted strain include increased time necessary for coagulase to clot serum and a loss of β -lactamase activity. Therefore, mutant cells become susceptible to β -lactam antimicrobials while demonstrating resistance to vancomycin. It has been hypothesized that these changes may allow *Staphylococcus aureus* to survive in the presence of vancomycin by binding the antimicrobial while allowing cell wall synthesis to continue unaltered.