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XI CONGRESO MUNDIAL DE BÚFALOS

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Memories THE 11th WORLD BUFFALO CONGRESS

Memorias del XI Congreso Mundial de Búfalos Cartagena, Colombia, 2016

Commercialization competitiveness and transformation

Effect of Process Conditions and Buffalo Milk Powder Properties (*Bubalus bubalis*) Obtained by Spray Drying

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The buffalo milk river *Bubalus bubalis* is the second most produced in the world, being considered compared to other species as a milk high compositional, nutritional value and high technological potential, qualities that have allowed each even better position in the Colombian market. Currently spray drying is one of the techniques used for drying heat-sensitive materials and widely used commercially for milk powder. The objective of this research was to evaluate the conditions of spray drying technology for buffalo milk powder laboratory scale. A central composite design was used with four factors: (A) Level of fat (6.34, 4.65, 0.93%), (B) Inlet temperature air (160, 180, 200 °C), (C) Outlet temperature air (75, 80, 85 °C) and (D) Atomization speed (25000, 30000, 35000 rpm). The drying process is optimized using the methodology of response surface where process conditions and functional properties of the powder obtained were evaluated, being the response variables that showed statistically significant differences ($p < 0.05$) % Yield, Water Activity, % Solubility and % fat content. Additionally, was evaluated % Humidity, Peroxide (mEq.O₂/kg oil) and % effective recovery, which variables were not significantly different ($p > 0.05$). In general, the optimal conditions for spray drying suggested for obtaining powder according to the factors found were: Level of Fat (2.92%), inlet temperature air (184 °C) Outlet Temperature Air (83 °C) and atomization speed (23,463 rpm). The study showed that the drying conditions imposed can produce a stable milk powder buffalo milk, characterized by high nutritional and functional value, low-humidity, remain viable for a subsequent operation on a larger scale.

Keywords: buffalo milk powder, fat, water activity, Solubility, spray drying, Peroxides value.

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Comparte



The traceability system in the Italian buffalo supply chain

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This paper describes the development of a on line system for recording traceability of milk production and buffalo cheese, applied to the whole national territory after the publication of Decree 9 September 2014. Online system of traceability on buffaloes production has been proposed to Mipaaf and the Ministry of Health, applied to the whole national territory and managed by IZS del Mezzogiorno and by Sian in applicative cooperation, and implemented also by DQA and AIA. Buffalo breeders, cheese factory and intermediaries have the obligation to inform the platform on a daily basis and no later than the first two days of the week following the detection, respectively : a) The daily quantities of bulk milk and receivers; b) The quantities of buffalo milk and other buffalo feedstock, also in frozen form, purchased for the production of processed products, as well as the names of individual sellers; c) The quantities of products Mozzarella di Bufala Campana DOP and non DOP; d) The quantities of other processed products resulting from the use of buffalo milk; e) The quantities of buffalo milk and semi finished products stocked; f) The daily quantities of buffalo milk and other buffalo feedstock, also in frozen form, purchased with the indication of intermediary. Furthermore breeders must submit monthly production data for individual animal, taken on the first day of the month, but no later than 5 days after detection. A call center supports activities. Actually the production of 1667 farmers, 457 457 non DOP cheese farm; 103 DOP-Cheese farm- 35 non DOP intermediaries - 14 DOP intermediaries, are traced on whole national territory. The results achieved so far already provide interesting data on the production of milk and dairy products throughout the national territory and the inspectors use the system to make real-time checks.

Keywords: buffalo industry, traceability, information system.

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El gremio como agente de cambio en el desarrollo de la producción con búfalos en el país. Asobúfalos, un modelo a seguir

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Agremiarse, constituirse en un colectivo donde las voluntades se unan con un único objetivo ha sido por años la mejor forma de progresar. El objetivo del presente trabajo es mostrar la experiencia que la Asociación Colombiana de Criadores de Búfalos durante los últimos 10 años sobre la promoción de la especie en Colombia, basados en la cultura del conocimiento. Desde su creación en 1976 se dedicó a impulsar la especie, pero solo hasta inicios del 2002 entendió que el desarrollo de la producción debería basarse en el conocimiento de la especie. Fue así como se empezó un trabajo de generación de información. Su gran aliado inicial, fue el Grupo de estudio de Búfalos de la Universidad de Antioquia, posteriormente en el año 2007, el Ministerio de Agricultura y Desarrollo Rural financió la convocatoria Consolidación del sistema de registro genealógico y control lechero de búfalos y su impacto en la producción y el mejoramiento de los rebaños colombianos y posteriormente Pruebas de desempeño en baby búfalo y búfalos doble propósito, en procura de seleccionar los mejores individuos para características relacionadas a la producción y rendimiento de carne. Posteriormente la asociación entendió su papel como gestor de recursos y transferidor de tecnología y en el año 2013 formuló proyectos basados en el apoyo a los criadores asociados o no. Se ejecutó del proyecto "Mejoramiento sobre la competitividad, sostenibilidad y productividad de la producción bufalina en Antioquia" a finales del 2014, y el convenio N°20150904 realizado con el Ministerio de Agricultura y Desarrollo Rural- MADR con el que consolida la actividad de promoción de la producción con búfalos en el país, hoy en día se es miembro de otras asociaciones gremiales como Fedegan, Unaga, se tienen convenios con el ICA, y se tiene comunicación permanente con todos los entes de control.

Palabras clave: gremio, apoyo, información.

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Environment and climate change

Evaluation of thermal comfort in facilities buffalo in the city of Santarém – Pará

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Buffalo despite the adaptability to different environmental conditions, they have characteristics that make it a sensitive animals to sunlight and high temperature environments. The objective was to evaluate the thermal comfort in buffalo facilities in the city of Santarém-Pará using thermal comfort indexes. Were used six buffaloes, weighing 400kg. Environmental variables were verified using thermohygrometer and black globe thermometers and readings were made at 1 hour intervals. The facility was assessed at the dry bulb temperature (DBS) and relative humidity (RH), and thermal comfort inside was analyzed by BGHI. It was measured every hour surface temperature using infrared thermometer. To compare the environments as the DBS, RH, BGHI and surface temperature indexes was used completely randomized design: 24 treatments (hours), with 6 repetitions (stalls) for 2 days. Data were submitted to analysis of variance by the test "F" and the means were compared by the test Skott Knott at 5% probability. The temperature ranged from 25.33 to 34.52 °C, with values below those found in the literature for the upper limit temperature (36.1 °C). RH has changed between 55.08 to 89.92%, and remained within ideal conditions for the buffaloes from 13h to 17h (55-65%). The values for BGHI fluctuated between 75.07 and 84.18 getting above considered comfortable (74) at all times. The surface temperature varied from 29.43 to 35.65 °C, this increase can be attributed to attempt to eliminate heat of the animals. Even with temperatures below the upper limit of the analyzed facilities do not provide comfortable condition throughout the day, the high humidity modifies the conditions of thermal comfort, with increased BGHI, which interferes with the welfare of buffaloes and imposing a need for management practices to use the environment to minimize this condition.

Keywords: environment, bioclimatology, facilities.

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El búfalo en la rehabilitación de humedales: finca las delicias en Guanacaste, pacífico norte de Costa Rica

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Los humedales son muy importantes ecosistemas para la conservación de biodiversidad y debido a procesos naturales o humanos a menudo se encuentran afectados por el exceso de vegetación invasora (zacates, herbáceas y arbustos), las cuales saturan el paisaje y limitan las funciones ecológicas de dicho ecosistema, por lo que es necesario rehabilitarlos como parte de las estrategias para la gestión de adaptación y mitigación del cambio climático. El búfalo de agua (*Bubalus bubalis*), es un animal que ha sido y puede ser utilizado con éxito para el pastoreo en humedales ya que es el herbívoro con mejores características anatómicas y fisiológicas y con el mayor potencial para la gestión de vegetación en humedales tropicales, pues puede desplazarse con facilidad en terrenos húmedos y cenagosos, incluso pueden nadar y meterse hasta donde vacas y caballos no pueden llegar, porque se quedarían atascados. Este trabajo de investigación se ha realizado durante los últimos siete años en Bolsón de Guanacaste (coordenadas Google 10.350151, -85.418061), Costa Rica. Se delimitó un área de 30 ha de humedal la cual estaba invadida por Zarza, un arbusto espinoso impenetrable (*Mimosa pigra*), el Platanillo (*Thalia geniculata*) y zacates, sin presencia de aves acuáticas. Se cortó la zarza, se hicieron separaciones de 2 ha cada uno y se sometieron a un sistema de pastoreo rotacional con búfalos de agua, con 20 unidades de carga animal y un tiempo de estancia variable. Se evaluó periódicamente el porcentaje de vegetación controlada, el área de espejo de agua recuperado, y la diversidad y abundancia de especies de aves acuáticas, así como la condición corporal de los búfalos. Se encontró que mediante el pastoreo controlado y extensivo, los búfalos remueven la biomasa de plantas invasoras del humedal y exponen los espejos de agua en condiciones de barro expuesto, en los que se observa diversidad de plantas, semillas y pequeños organismos que son fuentes de alimento, lo cual es muy atractivo para las aves acuáticas cuya diversidad y abundancia se incrementó significativamente mientras que los búfalos mejoraron sus condiciones productivas y reproductivas. Por lo anterior se concluye que el pastoreo rotacional con búfalos de agua es una actividad de mucho interés para la gestión de vegetación invasora en humedales tropicales y la producción bufalera, pues los animales se desarrollan bien y producen leche y carne de alta calidad ecológica mientras hacen la función de máquina biológica que limpia la biomasa del humedal, por lo que es un modelo relevante y de interés para la conservación y la producción y como estrategia de gestión frente al cambio climático. Por lo tanto, al búfalo de agua se le puede atribuir la función de rehabilitador de humedales.

Palabras clave: búfalos, pastoreo, gestión de vegetación invasora, mitigación del cambio climático, condición corporal de los búfalos.

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Ethology

Target BCS at calving. A valid Indicator of Buffalo Welfare for different level of production in Mediterranean Italian Buffalo

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The goal of this study was to evaluate the 'target' Body condition score (BCS) of Mediterranean Italian buffalo during 270 d of lactation. BCS is a subjective measure of the amount of metabolizable energy stored in an animal. Change in BCS of dairy buffalo might be a good indicator of animal welfare, because its value indicate the extent and the duration of postpartum negative energy balance. Data for 54,785 BCS records collected by trained evaluators from 2004 to 2015, from the Italian Buffalo Breeders Association database, were used to determine the inter-calving profile of the BCS for animals with three different levels of production at 270d (L1: 1,300 kg \leq milk \leq 2,000 kg; L2: 2,001 kg \leq milk \leq 2,700kg; and L3: milk \geq 2,701 kg). BCS was evaluated using a continuous scale 0-9 points. The additive genetic relationship matrix included 456,588 animals of which 54,785 buffalo with records. BCS BLUP-estimates were obtained by including in the Animal Model the fixed effects of age-parity, days from parturition to BCS evaluation nested within the milk productive level of the animals, and herd-contemporary-group. The BCS-solutions for each level of production were analyzed with the linear regression model, where weeks of lactation, was the explanatory variable. Although it was found that the inter-calving profile of the BCS changes depending on buffalo milk production, there is a point at which animal with different production levels, reach the same value of BCS during lactation. This target BCS is equal to 6.87 and it is reached between 12 -13 weeks of lactation, a little later compared to the target BCS in dairy cows (10-12 weeks of lactation). Therefore, any change in BCS value at this stage of lactation, could be an indicator of the animal welfare and troubleshooting any causes of shifting the animal BCS from its target BCS.

Keywords: buffalo welfare, body condition score, Italian buffalo.

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Reference Range of Basal Body Temperature BBT in Buffalo and Animal Welfare

Range di riferimento della temperatura basale nel bufalo e benessere animale

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The aim of this work was the standardization of body temperature in Mediterranean Italian Buffalo. 100.000 temperatures were been detected over ten months, of which particularly 94.364 were considered in this study and that are which obtained between may '13 and February '14. Through these detections, body temperature of certain number of subjects was obtained. The individuality in physiological answering to basal temperature (BBT) balancing was showed both in an entire day and during the passage from summer go to the winter through to autumn. Two groups of data were collected: "ALL" and "CLEAN", referred to all data of all animal detected and the data cleaned of the individual temperatures out of range respectively.

The Authors of this research already using the important results of this study to compare body temperature and the physiological answers in reproduction. Considering that, the reproductivity in buffalo is the aim of several number of researches because of its rarity in seasonality and in the detection of heat.

Keywords: baseline, temperature, welfare, buffalo, heat.

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Implementación de técnicas de doma racional en el proceso de amanse y adiestramiento del búfalo para trabajo en Hacienda Bélgica, Maní, Casanare

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En la región de Los Llanos Orientales, el aumento significativo de los cultivos de palma de aceite africana, trajo consigo la implementación del búfalo como animal de trabajo para la recolección de fruto dentro de sus cosechas. Sin embargo el manejo de los búfalos de trabajo dentro de estas plantaciones, se convirtió en un inconveniente debido al desconocimiento del manejo de la especie y al maltrato generado por la implementación de animales no mansos y no adiestrados para estos procesos. Se requiere ofrecer a los búfalos las condiciones adecuadas de bienestar animal que permitan el buen desarrollo de su conducta, facilitando así el manejo e incentivando su docilidad mediante la doma racional, con la única finalidad de que el proceso de adiestramiento se realice con animales mansos que puedan adaptarse a este proceso sin dificultad. Se plantea como objetivo principal describir el proceso por el cual se seleccionan animales mansos, se implementa el proceso de adiestramiento y el proceso de introducción a las plantaciones de palma africana a través de técnicas de doma racional. Se emplearon 10 (diez) búfalos de 30 (treinta) meses de edad y 5 crías recién nacidas; a los cuales se les implementaron las técnicas de doma racional. Todos los animales desarrollaron el proceso de adiestramiento sin inconvenientes o malas conductas, finalizaron siguiendo las órdenes y aceptando los equipos de trabajo. Las técnicas de doma racional, donde se establece no infligir dolor al animal y un ambiente donde predominó el bienestar animal y la seguridad del mismo y de los operarios, garantizan la facilidad en los procesos, reducen el tiempo de amanse y adiestramiento; y aseguran que los animales no se pierdan por malas conductas dentro de las plantaciones de palma.

Palabras clave: Búfalo, Trabajo, Palma, Amanse, Adiestramiento, Docilidad, Adaptación.

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Welfare Impact of Heel Height on Claws Overgrowing of Mediterranean Italian Buffalo

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The objectives of this study were to detect the impact of heel height (HH) on: 1) claws overgrowing (CO), and 2) milk yield (MY) in Mediterranean Italian Buffalo, to improve animal welfare. Data of HH and MY were collected from 740 Mediterranean Italian Buffalo, in five herds, by two evaluators, in the year 2016. CO was reported for each animal. To detect the impact of HH on claws overgrowing, adjusted means of HH for buffalo that showed CO, and for animals that did not show CO, were contrasted using PROC GLM procedure of SAS (SAS, 2005). Whereas, for investigating the effect of HH on MY, estimates of MY for HH classes were estimated using MTDFREML sets of programs. The fixed model for the analysis includes age-parity, days in milk, and lactation year, as fixed effects; and random effect of residual. Solutions of MY for HH classes were fitted using non-linear regression model, where HH was the explanatory variable. Unadjusted mean for HH was 4.05 ± 1.20 cm; whereas, unadjusted mean for MY was $2,466.9 \pm 426.4$ kg. Buffalo with CO showed smaller ($P < 0.0001$) HH value (3.80 cm) compared to that of buffalo without CO (4.34 cm). Therefore, buffalo with $HH \leq 4.34$ cm tend to be more subject to claws overgrowing, compared to animals with higher HH. Non-linear prediction of HH solutions for MY, showed an increasing of MY up to HH of 4 cm. Then, MY decreased to 2,282.1 kg for HH of 8.5 cm. Since HH is an heritable trait, selection for buffalo with HH of 4-4.5 cm will reduce in the population the number of buffalo that suffer from CO. Selecting for HH of 4-4.5 cm will also improve milk yield in the population. In conclusion, HH trait will be considered in setting up the Buffalo Welfare index.

Keywords: heel height, buffalo welfare index, animal welfare.

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Ruminant Welfare®: development of a new approach to evaluate Buffalo Welfare, work in progress

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Animal welfare is a growing, compelling and urgent topic as a result of the interest that it generates among the citizens and consumers. The goal to be fulfilled is to define systems and protocols for assessing animal welfare that should be impartial, reproducible and science-based on risk analysis. On these premises, the Italian Ministry of Health has signed an agreement with the IZSLER, through the CRenBA and IZSM through the CRenBuf on development and management of a system to evaluation of welfare and biosecurity in buffaloes breeding system. The checklist development was based on the Risk Assessment Methodology as suggested by EFSA. Building on the CRenBA's work developed on the welfare dairy cow², this method is based on the analysis of two data groups: the assessment of the hazards (non-ABMs) occurring as a result of environmental conditions; the assessment of the risks, with the concerned adverse effects (ABMs), run by animals living in those environments. The system developed consists in 83 observations, listed in a multiple-choice checklist divided in five macro-areas: Farm management and personnel, Facilities and equipment, Animal based measures, Biosecurity, Alarm systems. The result of each area also provides an indication of the burden and importance of each of these on the final calculation of the animal welfare value. These check represent a functional and smart instrument to allow assign a numerical animal welfare index to each farm, and also, by the data collected in each Area, to supply at the veterinarians and breeders the tools to improve farm management and structures, respecting the farm's sustainability, and is preparatory to given to the development of a Ministerial trademark for animal welfare, giving answers to consumers and add value to the correct activities of the farmers.

Keywords: Buffalo, animal welfare, risk assessment.

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Nutrition, food and supplementation

Comparison of feeding systems for the fattening of buffaloes

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In native grasslands of northeast of Argentina, the buffalo reach weights of slaughter with 500 kg at 30 months of age. The purpose is expediting such process at 18 month. For this objective, we compare two feeding systems, T1) Feedlot, T2) Paddocks with feeders of self-consumption, and his influence over live weight gain (LWG), back fat thickness (BFT) and rib eye area (REA). From July 27 to September 28, 2015. Twenty six buffaloes with 337 kg, were distributed randomly in 3 pens of 80 m² and in 2 paddocks by 0.79 and 0.73 ha. The initial forage mass was 1.622 and 2.325 kg DM/ha, and the stocking rate was 7.6 and 11 buffaloes/ha. In T2, fed a ration with 83% whole corn and 17 % soybean pellet, CP: 14 %, EM: 3 Mcal/kg DM. In feedlot, after an habituation period, the diet was 12% rice straw, 73% whole corn and 15% soybean pellet, CP: 13%, EM: 2,93 Mcal/Kg DM. BFT and REA were determined by ultrasound in 18 buffaloes taken at random by treatment. Data were evaluated by ANOVA, and averages for LWG, BFT and REA were compared with Tukey test ($p \leq 0.05$). The intake was in T1 and T2 7.8 and 6.4 kg DM/head/day, without measuring the consumption of grass. The buffaloes in T2 had a weight gain significantly higher than T1, 1.585 vs. 1.137 kg/head/day. Differences in BFT and REA were not significant, 5.8 mm, 58 cm² for T1 and 6 mm and 60 cm² for T2. In both feeding systems is reached the fattening of the buffalo. The paddocks with self-consumption feeders were more efficient in terms of gain weight than the feedlot, possibly due to a faster adaptation period and higher animal comfort.

Keywords: feeding system, fattening, buffaloes.

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Nutritional characterization of buffalo milk production systems in the central region of Sao Paulo, Brazil

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References on the feeding management of dairy buffaloes are scarce and mean based on dairy cows. Studies in this area are practical relevance contributing to more efficiency of buffalo feeding systems. The aim of this study was to evaluate the nutritional characteristics of diets used in dairy buffalo, including chemical feedstuffs composition, types of roughage and concentrate, dry matter intake and milk yield. The study was conducted in four farms of dairy buffalo located in the central region of Sao Paulo, Brazil. Feedstuff samples used in eleven different diets provided by 794 lactating crossbred buffaloes were collected monthly as each diet, totaling 43 samples for different periods of lactation. The most common food in the diets was the brewery residue being used in 10 of the 11 diets with average 26.75% of the total diet, following by sugarcane chopped used in five diets (28.3% in the total diet). Concentrated feed was used in nine diets, (15.4% in the total diet). Characterized forages were four kinds in different proportions, ranging from 3.6 to 74.3% in diets. Buffaloes showed dry matter intake averaged 17.8 kg/animal/d, with 2.6% BW or 91 g/kg^{0.75} with minimum 13.4 kg (2.2% BW and 78 g/kg^{0.75}) and maximum 22.7 kilograms (3.2% BW and 113 g/kg^{0.75}). Individual average daily milk production was 8.1 kg, varying between 3.0 and 12.3 kg milk/animal and feed conversion of 2.2 kg of DM/kg milk produced. Nutritional milking buffaloes systems raised in the central region of São Paulo, Brazil is characterized by the use of high proportion of roughage (87%) using mainly brewery waste and sugarcane chopped, featuring diets with nutritional value to meet low and medium milk production. The data obtained may be used as references for lactating water buffalo, assisting in the process of formulating diets more efficient milk production.

Keywords: concentrate, feedstuffs, forage, roughage, ruminants.

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Replacement of corn silage for sugarcane silage diets on rumen metabolism in buffalo

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The quest for economic alternatives for feeding at low cost is essential for the sustainable buffalo raising. Sugarcane is a tropical grass with high dry matter production at low cost. This research aimed to evaluate the use of sugarcane silage in buffalo feeding on digestive physiology involving the rumen metabolism. Four adult rumen fistulated buffaloes were used in Latin Square (4 x 4) experiment with four periods of 28 days each. The treatments constituted of four diets with different levels of replacement of corn silage by sugarcane silage (0, 33.5, 66.5 and 100%) maintaining 70:30 roughage: concentrate ratio (grain corn and soybean meal). Degradability in situ of both silages (DM, CP, and NDF), pH, volatile fatty acid and NH₃-N productions were measured. Data were statistically analyzed according to GLM module in LS design. The replacements of corn silage by sugarcane silage has promoted an increase in ruminal pH, NH₃-N concentration, acetic (%M) and acetic:propionic ratio with decrease in propionic, butyric and total acid productions. No effect was observed in ruminal outflow rate and fluid volume among the treatments. There was an increase rumen potential degradability of DM and NDF in both silage samples in the buffalo fed on total sugarcane silage compared to exclusive corn silage. The insoluble potential degradable fraction (b) of CP in corn silage have increased using 33.5, 66.5 and 100% of silage sugarcane, but the number of *Entodinium* and total protozoa per mL of rumen contents have reduced sharply. The replacement of corn by sugarcane silage promotes significant changes in ruminal metabolism with worsening pattern of fermentation, but improves the degradability of roughage, probably due to rumen bacterial growth by reducing the number of protozoa.

Keywords: degradability, N-ammonia, Rumen protozoa, VFA.

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Impact of aging time on the quality meat from buffalo heifer

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Female slaughter is of great importance for the production of meat. However, one of the reasons the culling female is due to old age, which suggest that the meat of this category may have lower quality. Thus, technologies such as aging are very important, especially for the buffalo specie which is presented as more an alternative to high quality meat. The present study evaluated the effects of different aging times on the physical characteristics of buffalo heifer meat. Water buffalo heifers (n=10; 32-36 months old; Murrah breed) were slaughtered and samples of the Longissimus thoracis were collected. Samples were subsequently aged for 0, 7, 14, and 21 days, and analyzed for shear force (SF), myofibrillar fragmentation index (MFI) and muscle color. Orthogonal polynomial contrast statements were used to test linear and quadratic effects of different aging times using SAS 9.2, considered significant when $P < 0.05$. Linear reduction was observed in the SF versus aging time ($P = 0.0001$; $R^2 = 0.97$) while mean values of the MFI increased during aging ($P = 0.0003$; $R^2 = 0.92$). For color meat, no effect ($P > 0.05$) of the aging times was detected for lightness (L^*) and redness (a^*), however, the average values of yellowness (b^*) increased linearly by aging time ($P = 0.003$; $R^2 = 0.76$). Despite this increase in the yellowness, data shows that subjective perceptions of the difference in color of the buffalo heifer meat were barely perceptible to human eye ($\hat{I}^{\circ}E0-7 = 2.41$; $\hat{I}^{\circ}E0-14 = 2.28$ and $\hat{I}^{\circ}E0-21 = 3.02$). Thus, the aging process improves the quality of buffalo heifer meat which showed softer after the 21st day of aging. Furthermore, changes observed in coloration are not relevant for human perception.

Keywords: aging, buffaloes, color, tenderness.

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Comparison of live weight, thoracic perimeter and some biochemical and nutritional parameters, in buffalo cows and buffalo heifers from Corrientes, Argentina

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In some regions and under certain conditions, bubaline show best physiologic, productive and reproductive attributes than bovine. It is necessary to make clinic and laboratories research about this species. The aim of this work was to analyze weight differences, thoracic perimeter and some biochemical parameters, between buffalo heifers and buffalo cows from two different places of Corrientes, Argentina. A sample of sixty Mediterranean buffaloes from two farms located in Itatí town and the other in Empedrado were included in the study. A sample of 15 buffalo heifers and 15 buffalo cows were selected in each place. Live weight (LV) and thoracic perimeter (TP) were recorded, and blood samples were taken. Hematocrit, red and white blood cells and hemoglobin were measured using anticoagulated blood. Urea, total protein, albumin, total cholesterol, triglycerides and glucose were determined by blood serum, according to conventional laboratory techniques. Factorial arrangement design was used, where the place was the independent variable, and age was the treatment. The descriptive statistics and analysis of variance were performed by InfoStat (2016) software. Buffalo cows had higher LW and TP (575.72 ± 5.84 kg and 215.89 ± 2.35 cm) than buffalo heifers (409.10 ± 6.99 kg and 181.18 ± 2.81 cm). No differences were found in LW between places; however, there were differences in TP. Buffalo cows had higher values of hematocrit, red blood cells count, total protein, urea, total cholesterol and triglycerides than buffalo heifers. Whereas buffalo heifers showed higher values of white blood cells count and glucose. The highest serum levels from biochemical and nutritional variable were found in Itatí town. Several parameters showed significant interaction between age and place. We concluded that the residence place of animals established an important variation on blood parameters analyzed. In addition, age showed a significant effect on several of these parameters.

Keywords: blood, clinic, laboratories.

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Carcass characteristics of buffaloes in a feedlot and fed with increasing levels of concentrate in sugarcane based diets

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Research information about the sugarcane in buffalo feed are scarce. Sugarcane is tropical roughage, poor in crude protein but high in dry matter production. Is traditionally used in Brazil because its production coincides with the period of forage scarcity. However, sugarcane fiber has a low digestibility and may have a depressing effect on performance. Best animal performance has been observed using sugarcane as a roughage source in high-concentrate diets. The objective of this study was to evaluate carcass characteristics of buffalo fed sugarcane with different levels of concentrate. Twenty four buffaloes Murrah with initial body weight of 240 ± 20 kg and nine months old were assigned to completely randomized design. Animals were placed in individual pens where they received diets *ad libitum*, twice a day. The experimental period has divided into 30 d for adaptation and 84 d for observations and samples collection. Four treatments were used: 1) 80% sugarcane (SC) + 20% concentrate (C); 2) 60% SC + 40% C; 3) 40% SC + 60% C; 4) 20% SC + 80% C. The diets were isoproteics and urea/ammonium sulfate was used at 9:1 ratio to correct the protein level of sugarcane. The evaluated variables were hot carcass weight (HCW), cold carcass weight (CCW), weight loss during cooling (WLC), forequarter percentage (FP), hindquarter percentage (HP), pH 24 hours, temperature 24 hours and *longissimus* muscle area (LMA). A regression analysis was performed using the MIXED procedure. The treatments had no effect ($P > 0.05$) on WLC, FP, HP, pH 24 hours and temperature 24 hours. HCW, CCW and LMA increased linearly with the treatments. The elevation in the levels of concentrate promotes increase in the carcass weight and LMA.

Keywords: *Bubalus bubalis*, *longissimus* muscle area, *Saccharum officinarum*, supplementation.

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Ingestive behavior of buffaloes fed increasing levels of concentrate in sugarcane based diets

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From 2007 to 2008, the buffalo herd in the northeast region of Brazil increased by 12.5% against 0.5% for the cattle herd. Usually the buffaloes explorations are made under extensive systems and most of the time without the concentrated food. The forage supplementation is made very often in the worst feeding season, especially the sugarcane. The experiment was conducted to evaluate the ingestive behavior of buffalo fed sugarcane with different levels of concentrate. Twenty four buffaloes Murrah with initial body weight of 240 ± 20 kg and nine months old were assigned to completely randomized design. Animals were placed in individual pens where they received diets *ad libitum*, twice a day. The experimental period has divided into 30 d for adaptation and 84 d for observations and samples collection. Four treatments were used: 1) 80% sugarcane (SC) + 20% concentrate (C); 2) 60% SC + 40% C; 3) 40% SC + 60% C; 4) 20% SC + 80% C. The diets were isoproteics and urea/ammonium sulfate was used at 9:1 ratio to correct the protein level of sugarcane. The evaluated variables were time spent feeding (TSF), ruminating (TSR), idle (TSI), feeding efficiency of dry matter (FEDM), rumination efficiency of dry matter (REDM), number of meals (NM), duration of meal (DM) and duration of breaks between meal (DBM). A regression analysis was performed using the MIXED procedure. The treatments had no effect ($P > 0.05$) on TSF. However, the TSR and NM decreased linearly as the dietary levels of concentrate increased. TSI, DM and DBM increased linearly with the treatments. Higher levels of concentrate provide long cycles of satiety. On the other hand higher levels of sugarcane result in constraint, decreasing the intake and increasing the NM.

Keywords: feeding efficiency, idle, rumination, *Saccharum officinarum*.

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¿How much it is possible to further improve the quality of buffalo milk?

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The increase of unsaturated fatty acids concentrations in milk, healthier for consumers, makes it less stable, requiring a source of antioxidant. Thus it was aimed to evaluate the fatty acid composition of milk from buffalo fed diets with addition of flaxseed oil (rich in n-3) and vitamin E (dl-alpha tocopheryl acetate). Four crossbreed buffaloes (mean weight=655±37 kg, days in milk=94±36 and milk production of 6.4 kg.d⁻¹) were used in Latin square 4x4 in 2x2 factorial design (diet without oil, diet with flaxseed oil (25 g.kg⁻¹DM), diet without oil and with vit.E (375 IU.kg⁻¹DM) and diet with oil + vit.E). The diets (700 g.kg⁻¹DM of forage and 300 g.kg⁻¹DM of concentrate, 110 g.kg⁻¹DM of crude protein, 403.8 g.kg⁻¹DM of neutral detergent fiber and 676.9 g.kg⁻¹DM of total digestible nutrients) were supplied twice a day, the buffaloes were milked by hand in the morning. The determination of milk fatty acids was performed using gas chromatography. Results were analyzed using the GLM procedure of SAS 9.0. The concentration of n-3 was increased (P=0.01) in milk of buffalo fed flaxseed oil (3.94 vs. 11.16 mg.g⁻¹ lipid), reducing (P<0.01) the n-6:n-3 ratio (9.30 vs. 2.44). The concentration of n-6 was increased (P<0.01) by adding vitamin E in diet (20.14 vs. 23.93 mg.g⁻¹ lipid). The activity of Δ^9 -desaturase enzyme increased (P=0.03) the cis9-16:1/16:0 ratio and reduced (P<0.01) cis9-14:1/14:0 and cis9-18:1/18:0 with the addition of oil. Vitamin E also increased (P<0.01) the cis9-14:1/14:0 ratio. The addition of flaxseed oil and vitamin E in diets for buffalo improve the milk quality to the optimum values recommended by World Health Organization.

Keywords: quality milk, buffalo milk, buffalo.

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Calves feeding cattle the Blanco Orejinegro race with buffalo milk

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The forage supply is limited in some times of the year, therefore producers search for supplementation strategies in order to avoid weight loss or low growth rate and reproductive problems. A strategy used for calves is to withdraw them from their mothers in order to feed them with other nutritional sources; which allows a quick cow recovery for the next gestation. The objective was to compare the growth in the first 4 months of the "Blanco Orejinegro" males (M) and females (F) calves raised traditionally (Bon1) and the ones fed with buffalo milk (Bon2). The weight gains were different in the second and third month of life (Bon 1 M: 0.65 kg/d, F: 0.56 kg/d and Bon 2 M: 0.77 kg/d and F: 0.65 kg/d), greater on the Bon 2 males, which proves that the feeding strategy of BON cattle with buffalo milk notably improves growth in nursing stage.

Keywords: beef cattle, growth, milk cattle, nursing, nutrition.

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Sensory and microbiological characteristics of buffalo heifer meat subjected to different aging times

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The female slaughter represented approximately 50% of the total cattle slaughter, including slaughter of buffaloes, in Brazil. However, the advanced age of females of discard is responsible for lower meat quality of this category. Particularly, characteristics as tenderness is most affected. Thus, technologies such as aging are very important, especially for buffalo specie which is presented as more an alternative of meat of high quality. The present study evaluated the effects of different aging times on the sensory and microbiological characteristics of buffalo heifer meat. Ten water buffalo heifers of the Murrah breed, at 32-36 months old, were slaughtered and samples of the *Longissimus thoracis* were collected. Samples were aged for 0, 7, 14, and 21 days, and analyzed for sensory and microbiological characteristics. Orthogonal polynomial contrast statements were used to test the effects of different aging times. For sensory analysis, was used the Student t test (normal distribution) and Mann-Whitney test (without normal distribution), considered significant when $P < 0.05$. No effect ($P > 0.05$) of aging was detected for the sensory parameters of aroma, strange aroma, flavor, strange flavor, and juiciness. Only the average values of tenderness were different ($P < 0.01$), wherein the aged samples (day 21) were considered "soft", whereas the non-aged (day 0) meat was "moderately dry". No relationship ($P > 0.05$) between aging time and the total bacteria counts was detected. However, the psychrotrophic bacteria and enterobacteriaceae increased linearly with aging ($P < 0.01$). Despite this microbiological growth in aged meat, bacterial counts were within the limits deemed appropriate for consumption at all aging times. In conclusion, aging improves the tenderness of the meat of water buffalo heifers without changing the microbiological quality of the product.

Key words: aged meat, bacteria counts, buffaloes, tenderness.

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Supplementation of growing buffaloes grazing xaraés-pasture (*Urochloa brizantha* Syn. *Brachiaria brizantha* cv. Xaraés) during rainy season

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This study was conducted to evaluate the performance and carcass traits, of growing water buffaloes receiving supplementation or not supplementation on under rotational stocking on pasture. The objective of this study was evaluate 20 buffaloes [Murrah water buffalo, 10 + 1 month of age and 206 + 29kg body weight (BW)] growing on pasture (*Urochloa brizantha* cv. Xaraés) receiving supplementation (0.6% BW), or not (control) during rainy season. Animals were divided in two treatment groups (supplemented and control; n = 10 animals, for each treatment). Body weight was measured at the beginning and the end of the rainy season (180 days) to determining total gain (TG) and average daily gain (ADG). Rib eye area (REA), backfat thickness - 12th rib (BF), and rump fat thickness (RFT) were determined at the beginning (0d) and the end (180d) of the study by ultrasound technique. Data were analyzed with PROC MIXED. Results are reported as least square means (LSMEANS) according to treatment. Significance was set at $P < 0.05$. No treatment effects ($P = 0.64$) were detected for initial BW (202.45 vs. 208.75 kg for supplemented and control animals, respectively). Supplemented animals had greater final BW ($P = 0.03$), TG ($P < 0.01$), ADG ($P < 0.01$) than control animals (338.63 vs. 301.86 kg for final BW; 136.18 vs. 93.11 kg for TG; 0.76 vs. 0.52 kg for ADG, for supplemented and control animals, respectively). Effect of treatment was detected ($P < 0.01$) for REA180d, where supplemented animals showed higher values when compared to control animals (42.74 vs. 33.10 cm² for supplemented and control animals, respectively). No treatment effects were detected for BF180d ($P = 0.72$) and RFT180d ($P = 0.69$) (7.49 vs. 7.31 mm for BF; 5.46 vs. 5.18 for RFT, for supplemented and control animals, respectively). In conclusion, supplemented animals had higher values for performance. Supplementation during the rainy season is an option to improve the production of buffalo in growing phase. Supported by FAPESP #2014/06446-3.

Key words: growing phase, performance, water buffalo.

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Feeding frequency evaluation on ruminal metabolism in buffalo

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Buffalo can modify the digestive behavior according to the type, quantity, food accessibility and management practices. Feeding frequency must be suitable for improved production efficiency with the best practice to minimize production costs. This study aimed to evaluate the effects of daily feeding frequency in buffalo, involving ruminal fermentation, degradability and rumen protozoa population. Four buffaloes rumen cannulated were subjected to an experiment design in reverse (4 x 2) with 2 sub-periods of 21 days each in two treatments: feeding frequency daily diet once (FF1x) and four times (FF4x) provided to every 3 hours from 7:00 to 16:00. The animals were fed with corn silage (88%), concentrate ration and mineral supplement. Ruminal degradability in situ of corn silage (DM, CP, and NDF), volatile fatty acid and ammonia N productions, and pH in sampling times of 0, 2, 4 and 8 hours after the first feeding were measured. Data were statistically analyzed according to doubled reversal design by statistical program. The feeding frequency did not affect the kinetics of degradability except the degradation rate that was higher in FF1x. No effect was observed in outflow rate from rumen and ruminal fluid volume between both feeding frequencies. No effect was observed in rumen fermentation production, except in molar percentage of butyric acid. Also, feeding frequency did not affect the counting of Entodinium, Diplodiniinae, Isotricha and total protozoa per ml rumen content. However, the sampling time after two hours feeding has reduced the protozoa number in FF4x, but not in FF1x. Change of feeding frequency from once to four times a day does not promote significant changes in rumen metabolism buffalo. To evaluate the count and identification of ciliated protozoa in the rumen, it is recommended sampling before feeding.

Keywords: degradability, N-ammonia, Rumen protozoa, VFA.

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Lactation curve characterization depending on length and number of lactation and buffalo age fed on low positive dietary cation-anion difference (DCAD)

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Several factors affect the milk production and composition, including genetics, age, number and stage of lactation. These effects need to be properly characterized in the buffalo lactation curve to improve the adoption of appropriate management practices and the efficiency in production systems. This study aimed to characterize the lactation curve associated to duration, number of lactation and age of buffalo. Individual milk production were measured in 206 buffaloes with an average weight of 650 kg, for 365 days, fed diets with 85% forage and 15% concentrate and DCAD average of +83 mEq/kg DM. They were evaluated daily and cumulative production of milk in four periods (90, 91-180, 181-270 and 271-365 lactation days) and monthly daily production for the duration of lactation, lactation number and buffalo age. The data were analyzed using the PROC MIXED (repeated measures over time) and mean Tukey test ($P < 0.05$) (SAS 9.3). There was no difference in the cumulative production among lactation periods up to 90 days. No difference was observed in daily milk production until the 5th month of lactation among the different lactation length groups except during the 3rd month. From the 6th month, more persistent buffaloes produced more milk. There were differences in the monthly milk production due to the number of lactations and age of buffalo. The accumulated production until 90 days of lactation is independent of lactation period. The genetic potential of the persistency of lactation can be better evaluated when animals are between 91 to 180 lactation days. From beginning to 90 days of lactation, buffaloes in above 4 lactations or 7 years old produce more milk than newer buffaloes.

Keywords: Animal nutrition, milk production, ruminant production.

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Effects of α 1-casein variants on buffalo milk properties and Mozzarella cheese quality

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In this study, the milk samples were divided into two groups according to difference of α 1-CN variants and the other group was mixed milk sample (MMS). The objectives of this study were to evaluate the effects of α 1-casein allelic variations on the composition, texture and functional properties of mozzarella cheese. The milk composition, including freezing point, pH and physicochemical properties were compared in each group, besides, Mozzarella cheese was manufactured from each group and been measured the parameters of cheese yield, fat, protein, moisture, pH, titratable acidity, texture and microscopic structure. Results showed milk samples with AA type α 1-CN variant showed higher milk freezing point, non-fat milk solids content (SNP) and lactose content ($P < 0.05$) than BB type, while lower protein, fat and total milk solids ($P < 0.05$) than BB type, milk with two types of α 1-CN had higher titratable acidity and SNP ($P < 0.05$) and much lower fat and total milk solids than MMS. The milk with AA type of α 1-CN variant had shorter rennet coagulation time and pH than AB type. The Mozzarella cheese pH, melt ability ($P < 0.05$) of milk with AA type of α 1-CN were higher and much lower cheese production and free-oil than AB. The Mozzarella cheese had lower hardness, springiness, adhesiveness, chewiness ($P < 0.05$) but higher cohesiveness ($P < 0.05$) of milk with AA type of α 1-CN were lower than AB. The cheese made from type AA milk showed dense protein structure and many holes. In conclusion, the α 1-CN had a significant influence on milk traits and mozzarella cheese quality.

Keywords: Chinese buffalo, Mozzarella cheese, Polymorphism, Milk quality, α 1-CN.

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Productive and economical assessment of copper and zinc injectable supplementation in buffaloes at western Pará, Amazon

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The buffalo production in Western Pará is usually extensive and with low technological level, using wetland ecosystems that hinder mineral supplementation of livestock. Thus, this study aimed to evaluate the effect of injectable supplementation of Cu and Zn on the weight gain of buffalos in the Western Region of Pará, as well, to evaluate the economic viability of this supplementation. Eighty male, crossbreed, non-castrated buffalo steers were used weighing 303.7 ± 52.9 kg (200–418 kg), 18–36 months-old, that were blocked by age: young animals (18–24 months) and adult animals (24–36 months) and divided into two groups, control and treated. The animals were weighed after a minimum 15-hour fast in following moments: D0 (baseline); D40 (40 days after the start of the study); D80 (80 days); D110 (110 days) and D150 (150 days). In D0 and D80 treated group received subcutaneous injection (1 mL / 50 kg body weight) of a copper and zinc formulation (Suplenut®) and the control received the same volume of saline. The data were subjected to analysis of variance using PROC MIXED (SAS) for repeated measures, considering the effect of treatment, block, time and interaction. The animals of the treated group showed a significant increase ($P=0.0029$) in cumulative weight gain, influenced by time ($P=0.0001$) but without block effect ($P=0.1762$). As there was no interaction between time and treatment block ($P=0.1975$), thus the effects can be evaluated separately. The cumulative weight gain was influenced by greater weight gain for treated animals at D40 ($P=0.0285$). Supplemented animals obtained R\$29.36 additional net income, generating return on investment of 18.3%. Supplementation of copper and zinc with commercial product Suplenut® was efficient to increase the weight gain of young and adults buffaloes, being economically suitable for the buffalo herds in the western region of Pará.

Keywords: buffalo, economic return, Suplenut, weight gain.

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Production and management

Correlation of body weight and body measurements of buffaloes

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The Para state, located in northern Brazil is the largest producer of buffaloes in Brazil. In this regard, the determination of the buffaloes body weight is important to evaluate the growth and nutritional status, and establish the animal's sale value. However, the economic reality of farms in the Lower Amazon does not allow the purchase of analytical balances considered financially costly. Minervino *et al.*, (2008) studied the characterization of the productive system of Santarém livestock, and found that only 38.1% of the properties had balance, so alternatives such as body measurements to determine the weight of the animals become important. The aim of this study was to obtain body weight and height measurements at the withers height (WH), hip height (HH), body length (BL), pelvic length (PL), thoracic perimeter (TP), scrotal circumference (SC) and rump width (RW) and then the Pearson correlations between the weight and measures. Eighteen males were measured aged 2 and 3 years from properties in the city of Santarém-PA, with analytical balances. The Pearson correlation coefficients between the body measurements were calculated using the CORR procedure, with 1% probability. The results were: weight 329,74kg ($\pm 44,45$ kg); WL 127,42 cm ($\pm 8,02$); HH 126,84cm ($\pm 5,03$ cm); PL 320,82 cm ($\pm 9,86$ cm); BL 175,05 cm ($\pm 10,58$ cm); TP 170,16 cm ($\pm 11,1$ cm); SC 25,75 cm ($\pm 1,48$ cm) and RW 69,75 cm ($\pm 3,9$ cm). Among the measures analyzed only pelvic length and thoracic perimeter showed higher degree of association with body weight (0.865 and 0.832, respectively). We concluded that the thoracic perimeter (in the form of a zoometric tape) is an efficient way to estimate the live weight of buffalos in the absence of an analytical balance at the place where the animals are.

Keywords: biometric measurements; Pearson correlation; thoracic perimeter.

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Estimation of genetic parameters, breeding values and genetic trends for milk yield, calving interval and first calving age of buffaloes (*Bubalus bubalis*) in Tierralta – Córdoba (Colombia)

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It has been known for a long time that animals must be genetically evaluated and selected in the environment where they have to perform. However, most producers use as germplasm individuals selected in environmental conditions and with selection goals differing from local needs. The objective of this work was to estimate genetic parameters, breeding values and genetic trends for milk yield, calving interval and first calving age in the conditions of a buffalo production system in Colombia. Twenty-two years of records from a buffalo herd located in Tierralta – Córdoba (Colombia) were used. An animal model for univariate and bivariate analyses was fitted for first calving age (FCA), milk yield per lactation period (MY), and calving intervals (CI). Contemporary group and calving number were used as fixed effects for MY and CI, while only contemporary group was used for FCA. Estimated heritabilities (se) were 0.19 (0.03), 0.04 (0.02), and 0.26 (0.09) for MY, CI, and FCA, respectively. Genetic correlations (se) were 0.21 (0.03) between MY and CI, 0.23 (0.29) between MY and FCA, and 0.01 (0.60) between CI and FCA. Genetic trends were 0.38 ± 0.50 ($P= 0.47$), -0.38 ± 0.03 ($P<0.01$), and 0.10 ± 0.01 ($P<0.01$) for MY, CI and FCA, respectively. Heritability estimates for milk yield and first calving age suggest that in this population it is possible to select efficiently for these traits, however, the positive genetic correlation between them dictate that selection index methodology be used to reduce undesirable correlated responses. Estimated genetic trends indicate great potential for improvement in this population.

Keywords: *Bubalus bubalis*, genetic parameters, genetic trends.

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Grayscale histogram use for mammary development assessment of crossbred Murrah heifers

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The objective was to evaluate the mammary development of buffalo heifers through mammary ultrasound images analysis. Six crossbred Murrah females, 294.96kg of average weight, reared on extensive management were evaluated every 28 days, from 12 to 24 months. Grayscale histograms (GSH) were made from ultrasound images of the mammary gland (cranial and caudal) to determine echotexture which was homogeneous when the ratio of the number of the most repeated pixel in the image and the total number of pixels (NMOST/NALL) was higher than the pixel amplitude standard deviation (SD), and heterogeneous when this ratio is lower. The average of grayscale (LMEAN) indicates echogenicity. The lower the value, the lower the echogenicity. We used analysis of variance and Tukey 5%. There was statistical difference between months and mammary quarter, being the caudal the most significant results. The relationship NMOST/NALL ranged from 0.004 to 0.007; .005 to 0.009, SD 60.40 to 148.00; 91.00 to 154.00 and LMEAN 69.56 to 145.81; 86.84 to 152.85 for mammary glands cranial and caudal, respectively. Therefore, echogenicity was high and echotexture heterogeneous. Significant changes in pixel values suggest that through the GSH is possible to follow the dynamics of mammary development, especially parenchymal, whether heifers are pre- or postpubertal.

Keywords: mammary images, mammogenesis, dairy buffalo.

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Ultrasonography evaluation of buffaloes carcass quality of Murrah breed fed with different levels of spineless cactus as a replacement of the wheat bran

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The real-time ultrasonography is one of the most widely used techniques for in vivo prediction of carcass or body composition in animals. It is a non-invasive technique which quantifies different tissues in live animals. The objective of this study was to evaluate the correlation between traits obtained by real time ultrasound and those obtained in the carcass. Twenty non-castrated buffaloes of the Murrah breed with an initial body weight of 300 ± 20 kg were used. The animals were fed with different levels of spineless cactus (0, 33, 66, and 100 %) as a replacement of the wheat bran in a sugarcane-based diet. The measurements obtained by ultrasound in real time were determined using an Aquila PieMedical® ultrasound machine, equipped with a 3.5-MHz linear transducer, 24 hours before slaughter. The measurements taken were longissimus muscle area (LMA) located between the 12th and 13th ribs and subcutaneous fat thickness (SFT). The animals were slaughtered after 84 days of feedlot. The SFT from the carcass was evaluated using a pachymeter and the LMA was evaluated by using a quadrant grade ruler. The experimental design was completely randomized. It was observed a linear decrease effect on the LMA obtained by ultrasound and carcass. The coefficients of determination for LMA obtained by ultrasound and in the carcass were high and similar, proving that 96% and 98% of the total variation is explained by the replacement levels. The treatments had no effect ($P > 0.05$) on SFT obtained by ultrasound and in the carcass. The correlation between LMA obtained by ultrasound and in the carcass ($r = 0.96$) was positive and highly significant ($P < 0.001$) and the same occurred to SFT ($r = 0.94$). Ultrasound can be an accurate estimator of carcass traits in buffaloes of the Murrah breed.

Keywords: *Bubalus bubalis*, fat thickness, longissimus muscle area, ultrasound.

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Welfare implications of buffalo calves

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The EU legislation do not contemplate the Welfare of buffalo calves, because it was made up for the welfare of bovine calves (Directive 119/2008/CEE). However, there are important differences between the two species, buffalo vs bovine that can impact the welfare of buffalo calves. The EU legislation (Directive 119/2008/CEE) indicates that bovine calves must assume feed that supply a content of hemoglobin of at least 4.5 mmol/liter of blood. For this reason, a daily dose of fiber must be assumed by each calf after the second week of life. Since rumen development in buffalo takes more time to complete, compared to that of bovine, introduction of hay in the buffalo calves diet should occur later than that regulated. There is high incidence of gastro-intestinal diseases, by which veal buffalo are contagious from adult's feces or from other veal buffalo's feces. Rumen development for the veal buffalo occurs later compared to that of veal bovine. Also, the productive cycle of buffalo is longer than that of bovine. To avoid the infections of veal buffalo via faces, buffalo calf are confined inside galvanized cages, up to two months of life (EU Directive 119/2008/CEE). However, to reducing veal buffalo mortality, a longer period inside the cage should be evaluated (up to three months of life). The width and the height of the individual pen, should be suitable to the veal buffalo conformation. The three dimensions of the individual pen for buffalo calves up to three months of age should be 100x150x190.

Keywords: animal welfare, buffalo, buffalo calves.

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Background and situational diagnosis of raising buffalo (*Bubalus bubalis*) in Valle Sacta, Cochabamba, Plurinational State of Bolivia

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Buffalo (*Bubalus bubalis*) is a species that, in recent years, its importance has increased due to its ecological benefits in terms of adaptation and adaptability to tropical areas, also for its nutritional qualities of milk and meat. Currently buffalo occur in the Fundo Valle Sacta, Race Tropical Agriculture and Renewable Resources Management, Faculty of Agricultural and Pecuary Sciences of the University of San Simón (UMSS) department of Cochabamba, Plurinational State of Bolivia. The purpose of this paper is to report the background and analysis of the situation of these buffaloes, raised in an extensive system of grazing native pastures mostly with cattle on an area of 40 hectares, located mostly in and low-lying areas. Such breeding is managed for conservation, production, research and social interaction. From 1978 to date Mediterranean buffaloes are raised. Twenty five to thirty five discards bufalinas units are sold annually, milk and cheese Catren type (cheese pressing unripe), are also sold to generate resources for the Academic Unit of Valle Sacta. Since 2013, there have been an important development in research specially focused on management issues, food, health, agro-tourism and traction. This relevant development was possible with the help of researcher-professors and undergraduate students. The new information obtained throughout research is used as teaching material, and the participation in fairs encourages the production of buffalo in the area. The current population of buffaloes in the Fundo Valle Sacta is 95 buffaloes. The exemplary management and health that is performed is similar to cattle. As for milk production are obtained usually 1.5 to 2 liters / buffalo / day, and in some cases up to 4 liters on extensive system with native grass and supplementation based *Creatilia* sp., *Axonopus escoparius* and *Musa paradisiaca*. With 10 liters of milk are processed cheese 1.8 kg and 1.5 kg Catren type mozzarella cheese. As for meat production, channels with average weights of 125 kg of bubillos a year and a half old were obtained, fed only pasture. As for health preventions against FMD, clostridiosis and rabia are made similar of bovine. With regard to parasitic diseases we have verminosis in calves, being *Neoscaris vitolorum*, *Strongyloides* sp. and *trichostrongyloides*. External parasites are present in the dry season (July to November). In addition sucking lice they are *Haematophinus eurysternus*, and *Dermatobia honminnis* furunculosis causing papules with pores in the epidermis. Finally, in terms of innovative activity in Agro-tourism it will be implemented together with the Autonomous Municipal Government of Puerto Villaruel and breeding program in dairy buffaloes fitness medium and long term.

Keywords: water buffalo, diagnosis of the situation, production.

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Influence of external morphological characteristics of lactating buffaloes udder on the performance of calves in the first months postpartum

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Morphological knowledge is necessary to develop technologies for a better use of dairy buffaloes. In this sense, this paper aims to describe the main phenotypic characteristics of udder and teats of crossbreed Murrah buffaloes and relate them to their offspring performance. We used six multipara 5 year old buffaloes. Every 14 days, during the first 2 months of lactation, measurements of udder and teats were taken, using a centimeter (cm) graduated tape. From udder insertion to the edge of the teat (udder length), from vulva base to the beginning of the udder (udder height) and the rectilinear distance of udder insertion (udder width) and from the beginning of the teat to its end (teat length). In the same period, offspring body weight (kg) was determined. Data were analyzed by Pearson correlation and was considered a 5% significance. Udder width showed a negative and significant correlation with offspring body weight ($r = -0.50$; $P = 0.004$). The other correlations between morphometric characteristics of udder and teat were not significant ($P < 0.05$). Average udder width found in this study was 23.18cm, indicating large udders, which provide greater storage of milk. However, the increase in body performance of calves was associated with decreased capacity and volume of milk produced. Therefore, important adaptive mechanisms probably contributed to the maintenance of the dynamic state of the gland during this period, reflecting the close ratio between body weight gain, milk production and mammary development.

Keywords: mammary gland, visual evaluation, udder measures, dairy buffaloes.

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Influence of body status of primipara buffaloes on offspring weight at birth

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The assignment of body condition scores (BCS) is one of the way to monitor nutritional status in water buffalos. In pregnant females, a review of this information can predict the potential return of reproductive activity and the suckle capacity after delivery, so the evaluation of such data proves to be crucial. The objective of this study was to quantify the connection between the mother nutritional status at parturition with their neonates at birth. For this, were assessed at delivery, weight (kg) and BCS of 6 pregnant crossbreed Murrah buffaloes and the weight of their offspring. BCS has been determined by a single rater and was based on visual and tactile assessments of body reserves at specific spots of the animal body, using a biological scale of 1 (very lean) to 5 (very fat), 0.5 subunits. The animals were kept in paddocks with water fountain, trough and pasture formed by *Brachiaria brizantha* cv. *Xaraes*; submitted to rotational grazing, mineral salt ad libitum. Data were analyzed by Pearson correlation, considering 5% significance. Average weight and mothers BCS were respectively: 493.50 and 2.50 and average weight of pups was: 44.00. There was no correlation between the mother weight and the neonate weight ($r = 0.38$; $P = 0.457$), and the same similarity was observed between BCS of the mother and the weight of offspring ($r = -0.62$; $P = 0.181$). Note that the mother body reserves and the weight of offspring were satisfactory, indicating that the nutritional management was well executed and that the animals responded adequately to this management, acquiring nutritional status to meet the nutritional pregnancy requirements without noticeable negative impact on the offspring.

Keywords: body condition score; mobilization of body reserves; nutrition; Murrah buffaloes.

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Hemodynamic evaluation of mammary development and its relationship with body performance crossbred murrah buffaloes during pregnancy

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The aim of the study was to evaluate hemodynamic parameters of the mammary arteries and relate them to the body weight gain of buffaloes during pregnancy. We used 6 pregnant crossbred Murrah buffaloes 24 months old. Every 28 days, Doppler ultrasound of cranial and caudal mammary arteries was held and determined resistivity index (RI), pulsatility index (PI) and internal vessel diameter (ID) and, additionally, animals were weighed and given the body weight (BW) expressed in kilograms (kg). The animals were evaluated from the first to the last month of pregnancy. For statistical evaluation of hemodynamic indices, we used analysis of variance, considering repeated measurements over 12 months and Pearson correlation, and considering $P < 0.05$. There was a progressive decrease in RI and PI values and an increase in ID in both mammary arteries when the first was compared to the last month of the study. However, the left mammary caudal artery showed a significant difference to the hemodynamic values, being observed lower RI ($P < 0.0001$) and higher ID ($P < 0.0001$), in the five and three last months of the trial period, respectively. Significant correlation was found, high and positive between RI and PI ($r = 0.83$; $P < 0.0001$) and significant, high and negative of RI with DI ($r = -0.74$; $P < 0.0001$) as well as significant, moderate and negative PI with ID ($r = -0.66$; $P < 0.0001$). BW had linear effect on RI ($R^2 = 0.44$; $P = 0.0019$) and PI ($R^2 = 0.35$; $P = 0.0013$) and quadratic influence of BW on ID ($R^2 = 0.59$; $P = 0.0001$). It was found that significant hemodynamic changes at the end of pregnancy infer the possible increase in the adaptability of the mammary tissue, according to the metabolic demand of the gland. Therefore, hemodynamic indices vary with the physiological state and they may be considered as reference for the evaluated species.

Keywords: dairy buffaloes, Doppler, mammary blood flow, body performance.

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Management features and productivity in Latium buffalo farms

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Italian buffalo breeding is intensive. Breeding systems based on balanced feed administered in confinement, require a particular attention to livestock density and feed and water availability to allow resources accessibility reducing competitions and injuries. The aim of the present study was to verify these features in Latium buffalo farms. 43 farms were visited during the summer months. In those farms the size (total number of animals) and the mean production per head (kg/head/day) were recorded. The farms were considered as big over 150 heads, while the production was considered as high when equal to or exceeding 8 kg/head/day. The total space allowance (m²/head), space at the manger (cm/head) and watering space (cm/head) were evaluated in the lactation group. A space allowance below 7 m²/head was considered as negative for the welfare, as well as for the milk hygiene; in the same way drinking frontages shorter than 7 cm/head were considered as negative; and concerning the space at the manger it was negative if there was a single front and no access to pasture for at least two months/year. Statistical analysis was performed using STATA. Mean farm size resulted to be 210 heads and mean production was 7.6 kg/head/day. The farms classified as big resulted significantly the most productive (P<0.001). Most of the farms (77%) obtained a positive assessment for the space allowance but only the 49 and 30% respectively for troughs and drinkers. In particular, differently from the bigger, most of the little farms obtained a positive result regarding the space at the manger because of sending the animals to the pasture, but it was not significant. Actually buffalo farmers are conscious about the importance of stocking density but still do not care about number and location of feeding and watering facilities that are crucial especially during lactation.

Keywords: *Bubalus bubalis*; milk production; farm size; housing; feeding.

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Study of milkability in Mediterranean Italian Buffalo cow raised in central Italy

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Milk production and milk flow profiles are important parameters to be recorded and evaluated as the practical use of these informations could be applied for breed selection program and/or for routine milking control at herd level. The aim of this study is to analyze the principal milk flow traits and milk yield, recorded in Mediterranean Italian buffaloes, raised in the Lazio Region (Central Italy). The investigation was conducted from 2005 to 2015 on 3,191 Mediterranean Italian Buffalo cows from 184 different Buffalo herds raised in Central Italy. The milkability was performed using 3 electronic portable milkmeter "LactoCorder" (WMB AG). The results of milkability for each herd were obtained using the specific program (LStat). Average milk yield was 3.86 ± 0.92 kg/head for milking session, while maximum flow and average flow were 1.31 ± 0.30 kg/min and 0.82 ± 0.16 kg/min respectively. Average total milking machine time was higher 10.35 ± 2.07 min, respect the time of milk ejection: the principal milking time was very low with only 4.24 ± 0.09 min., lag time 2.15 ± 0.95 min, and stripping time 0.23 ± 0.29 min, respectively 41.41%, 20.79% and 2.20% of milking machine time. Results showed that average blind milking was very higher (3.80 ± 0.17 min) and predominant when compared to other phases; this point is crucial for farmers because it increases management costs, and mastitis risk. Results showed some irregularity that indicate the need to always apply a proper udder pre-milking stimulation to reduce the milk let-down phase and total milking time. Moreover it is essential to reduce the Blind Phase at the end of milking to avoid teat stress, this practices could reduce susceptibility to mastitis and labour costs.

Key words: herd level, LactoCorder, milk flow.

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Relación del tamaño corporal de búfalas con desempeño productivo y reproductivo en la Bufalera Riomanso

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El propósito del estudio fue evaluar la relación del tamaño de búfalas mestizas con su desempeño productivo y reproductivo en el rebaño Riomanso ubicado en la Dorada, Caldas (zona de bosque húmedo tropical de la región del magdalena medio colombiano). Se analizó la información productiva y reproductiva del rebaño de cría destinado a la producción de bucerros para ceba. Se estableció una base de datos con ayuda del software ganadero TP, en el periodo comprendido entre enero de 2004 y julio de 2014, evaluando información de búfalas multíparas (n=77). El análisis estadístico incluyó estadística descriptiva y análisis de correlaciones múltiples. Se obtuvieron promedios de 1,43 ±0,4 cm de altura a la cadera, 579.8 ±61 kg de peso vivo, 250.8 ±20 kg peso ajustado al destete (9 meses), 444 ±68 días de intervalo entre partos, un índice productivo promedio (peso búfala / peso destete) de 43.65 % e índice de vaca promedio (IV= IEP/peso al destete) de 53.46. El peso corporal se correlacionó con el peso al destete (r=0,22, p<0,05), la altura a la cadera de la búfala mostró una correlación moderada con el peso al destete (r=0,27, p<0,05), indicando que el tamaño de la búfala tiene influencia con el desempeño productivo al destete. La altura a la cadera se correlacionó moderadamente con el intervalo entre partos (r=0,40, p<0,05), sin embargo el peso corporal no estuvo correlacionado con IEP en las búfalas, estos hallazgos indicarían que la altura a la cadera estaría influyendo más en desempeño productivo y reproductivos que el propio peso del animal. El tamaño de búfala que se encontró más eficiente para el rebaño de Riomanso fueron las hembras medianas, en el rango de 140 a 145 cm, ya que estas fueron las que presentaron mayor productividad y mejor desempeño reproductivo.

Palabras clave: altura corporal, eficiencia, hembra, producción.

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Evaluación de la calidad de la información en 35 hatos bufaleros del país

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El gran cambio que ha tenido la producción animal, ha sido moverse de la cría de animales y posterior beneficio hacia establecimiento de sistemas productivos, que llegan hasta la completa automatización de la mayoría de procesos. El presente trabajo tiene como objetivo describir el estado del manejo de la información hatos bufalinos localizados en diferentes regiones del país. En este trabajo prospectivo se analizaron los registros correspondientes a la información colectada por los técnicos de la asociación que participaron en el programa de inseminación artificial, realizado en 5 departamentos del país durante la temporada reproductiva 2015-2016. Se tomó información sobre la identificación de los animales, raza, estado fisiológico, condición corporal, edad, producción de leche, días abiertos. Adicionalmente se registró de cada predio la localización. Se evaluaron 1162 animales, localizados en 34 bufaleras, de 5 departamentos del país, Antioquia (15%), Caquetá (21%), Córdoba (29%), Magdalena (5%), Santander (29%) y Sucre (2%). Se encontró que el 100% de los animales estaban identificados, de estos 7,4% poseían el dispositivo de identificación electrónica nacional (DIN), el 86,2 % de los animales fueron caracterizados como mestizos, registros de producción láctea se obtuvieron en el 35% , días abiertos el 60%, paridad 46,2%, se pudo establecer la edad en el 71%. Es de resaltar que el 100% de los animales está plenamente identificado y se puede hacer su caracterización racial, principio fundamental del programa de mejoramiento, se debe resaltar el bajo número de animales 86 y de predios, 11,7 % (4/34) que habían puesto el DIN. La información relacionada con el manejo del hato, ha de ser mejorada, pues está alrededor del 50%, mejorar la información significa ampliar la cantidad de registros que debe ser analizada como insumo principal para la toma de decisiones.

Palabras clave: información, hatos, manejo, calidad.

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Measuring the Adoption of Improved Feeding Practices by Smallhold Dairy Buffalo Farmers in Nueva Ecija, Philippines

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A face-to-face individual interview-survey of 311 smallholder-farmers in the province of Nueva Ecija, Philippines was conducted to measure and analyze their adoption of improved feeding practices (IFPs) for dairy buffaloes, as introduced in previous technical trainings by the Philippine Carabao Center (PCC). The IFPs included feeding of improved forage, legumes supplementation, and feeding of concentrates. Dichotomous (yes or no) frequency and percentage responses along five stages, i.e., "awareness", "interest", "evaluation", "trial", and "adoption" were transformed to sigma (Z) scores for adoption. Frequency responses for "number of years of adoption" were likewise transformed to sigma scores. The two sigma scores were added to get the total adoption scores for each IFP. The total or combined adoption scores (dependent variable) for all three IFPs were then tested for linear correlation and multiple regression with selected socio-economic traits, farm characteristics, and other independent variables. Of the ten variables that showed significant linear correlation with the total adoption scores, only five were found to be significant predictors, namely "years of formal schooling", "years of experience in dairying", "animal inventory", "access to information, education and communication materials", and "number of agencies sought for technical assistance". Among the significant variables, the latter emerged as the most powerful predictor for total adoption scores. This would indicate that farmers adopt IFPs as they increase their social capital or communication network by linking with more agencies or institutions that are sources of information, technologies, and technical assistance on dairy buffalo management. Applying the same approach in measuring adoption level for other technologies in dairy buffalo management, as introduced by the PCC, was recommended.

Keywords: feeding technologies; sigma scores; socio-economics.

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Reproduction and genetics

Effect of body condition score at mating on the rate of pregnancy in buffalo

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In North East of Argentina, buffaloes breeding take place in waterlogged grasslands with low quality forage, especially in winter. Between weaning in December up to the beginning of the mating season in April, the female buffaloes must retrieve body reserves, so that this variable does not influence on the rate of pregnancy. The objective was to determine the relationship between the body condition at mating and the rate of pregnancy. The experience was made in 2014 and 2015, with 217 lactating buffaloes Murrah, Mediterranean and their crosses, belonging to three herds. The influence of the body condition score (BCS: scale 1 to 5) at mating on the pregnancy rate, was determined by Chi square and regression analysis. The BCS at mating had a significant effect on the pregnancy of the buffaloes ($p < 0.0001$). The equation, $y = 136 - 169/x$, where $y = \% \text{ pregnancy}$, $x = \text{BCS}$, explains the variations in the rate of pregnancy in response to body condition. The body condition 3.5 gets a pregnancy of 86%, so this could be considered the value threshold reproductive success in the adult buffalo. Although, buffalo is adapted to low-quality rangelands, the need for appropriate nutritional management is important before mating season. It is concluded that body condition at mating, is a variable that explains the reproductive efficiency of adult buffalo. The highest rates of pregnancy are accomplished with a body condition equal to or greater than 3.5.

Keywords: body condition, buffalo, pregnancy in buffalo.

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The Comparison of Lactation Curve with Different Models in Mediterranean Water Buffalo Raised in Istanbul

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This study was aimed to investigate biometry of lactation curve for Mediterranean water buffalo imported from Italy raised in private farm in Turkey. Total 72 head Italian water buffalo were used at first lactation and three calving seasons as animal material. Wood, Wilmink and Cobby and Le Du models were chosen in present study. The general average lactation period, total lactation milk yield average and average daily milk yield were found 234 days, 1607.4 kg and 6.86 kg, respectively. Determination coefficient was calculated for Wood, Wilmink and Cobby and Le Du models for summer calving season as 0.94, 0.92 and 0.93, respectively. Wood model was found the highest coefficient of determination in general. Moreover, persistency (S) and maximum milk yields (Ymax) for Wood model were calculated. These values were found as 5.89 and 9.76 for first lactation in general group, respectively.

Keywords: wood, wilmink, cobby and le du models, italian water Buffalo, lactation curve.

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Use of insulin and bovine somatotropin in artificial insemination in buffaloes protocols

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Introduction and objectives. The protocols of artificial insemination increasingly undergo changes to rely less and less heat detection and to increase the percentage of conception, giving rise to Artificial insemination in time fixed (IATF), which consists of a hormonal scheme that allows insemination in the opportune moment to ovulation. *Materials and methods.* Two tests were performed: the years 2014 (E1) and 2015 (E2); applying the Ovsynch Protocol and the use of insulin (Humilin R[®]: In) and somatotropin (Lactotropina[®]: bST). Used 44 buffaloes (2014); E1: 22 with In and bST and 22 without In and bST, and 50 buffaloes (2015); E2: 25 with In and bST; and 25 without In and bST; all the buffaloes with a postpartum period between 42 and 45 days and body condition between 4 to 4.2 (scale: 1-5); were randomly divided into two groups: group In which was applied (0,2 UI/kg, subcutaneous) on day 9 post insemination and equal to 500 mg via subcutaneous bST (Lactotropina[®], Elanco) five days prior to the first dose of GnRH and another dose at the time of insemination; and another group without In and bST was applied 6 ml of physiological saline, via subcutaneous placebo. The semen used for the two trials was the same; artificial insemination was carried out 18 hours after the last injection of GnRH by the same technical inseminador. 42 days post insemination were diagnosed through rectal palpation and ultrasound examination. The percentage of conception was compared using Chi-square test. *Results.* The results were significantly different ($P < 0.01$); 68.18 vs 54.54; 68.00 vs 52.00; % of conception for the years 2014-2015 for E1 and E2 respectively. *Conclusions.* It is concluded that the application of In and bST in buffaloes produces a favorable effect on the percentage of conception in protocols of IATF.

Keywords: buffalo, insulin, somatotropin, ovsynch, conception.

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Puberty age in mediterranean buffalo heifers in Corrientes, Argentina

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The objective of the present study was to determine the age of puberty through levels of progesterone ≥ 1 ng/ml, using a herd of 30 heifers buffalo in a pasture system, born and wean in 2014 and test in February and July of year 2015. The data registered was weight at birth (WB), weight at wean (WW), and weight at puberty (WP), daily gain preweaning (DGW pre), daily gain postweaning (DGW post). The blood was recovered from the jugular vein every 14 days to obtain serum for progesterone (P4) analysis by chemiluminescence. The puberal animals were divided every fifteen days according to their calving in: first (G1), second (G2), and third (G3). There were performed multiple measures using ANOVA, taking as a qualifying variable the calving through Duncan Test with an 5% α . The results for G1, G2 and G3 were WB 35.7(± 1.07), 37.6(± 1.11), 37.6(± 1.43) respectively ($p > 0.05$); WW 233.6(± 6.44), 242.4(± 6.65), 207(± 8.68) only significant ($p < 0.05$) G3 respect to G1 y G2. DGW pre, was 0.622(± 0.05), 0.830(± 0.05) 0.857(± 0.07), where G1 was different ($p < 0.05$), not so in DGW post that was 0.220(± 0.03), 0.248(± 0.03), 0.198(± 0.04) ($p > 0.05$). The P4 was 1.65 (± 0.52), 2.45 (± 0.52), 2.51 (± 0.65), being not significant ($p > 0.05$). For the age the results were 450(± 14.9), 412(± 14.9), 378(± 18.7) being different G1 y G3 ($p < 0.05$), and WP was 300(± 12.9), 297(± 12.9), 253(± 16.2), being different G3 ($p < 0.05$). These results indicate that puberty is reach at 413 days of age with 283 kilograms in weight for all groups.

Keywords: progesterone, weight, wean, puberal.

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Correlation between testicular morphometric parameters in Mediterranean bulls buffaloes in the northeast region of Argentina

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In male buffalo is possible that body and genitalia development may reach his full growth similar that bovine species, depending on selection and management in different stages (Vale, 2011). The objective of this study was to evaluate the correlation between age of the animals, body weight and biometrical testicular values. The 39 Mediterranean male buffalo were divided into three groups **G1**: 27 animals of 18 months of age, **G2**: 7 animals of 24 months of age and **G3**: 5 animals of 36 months of age; all of them located in the northeast of Corrientes. It was evaluated scrotal circumference (SC); height (HT) and testicular width (TW) of every testicle; morphometric measures, torax perimeter (TP), height to cross (HC) and sacrum (HS) and body weight (BW). The statistical analysis was performed by ANOVA, taking as a qualifying variable the ages and correlated them with ages, testicular and morphometric measures. The results of the analysis were significant according to the ages ($p < 0.05$) for SC, $G1 = 19.7 (\pm 2.2)$, $G2 = 24.9 (\pm 3.9)$, y $G3 = 29.7 (\pm 1.5)$; HT, $G1 = 5.5 (\pm 0.8)$, $G2 = 6.6 (\pm 0.5)$, $G3 = 8.3 (\pm 0.2)$. The measure for HT was significant for $G3 = 14.8 (\pm 1.5)$, not so for $G1 = 10.3 (\pm 1.5)$ y $G2 = 11.7 (\pm 1.4)$ ($p > 0.05$). As to morphometric measures the results were significant for the three groups ($p < 0.05$) HC, $G1 = 119.2 (\pm 4.5)$, $G2 = 129.7 (\pm 3.8)$, $G3 = 142.2 (\pm 3.1)$; HS, $G1 = 123.3 (\pm 4.7)$, $G2 = 136.4 (\pm 3.4)$, $G3 = 143 (\pm 2.2)$; TP, $G1 = 163.9 (\pm 7.3)$, $G2 = 184.1 (\pm 3)$, $G3 = 211.6 (\pm 5.2)$ y BW, $G1 = 327.8 (\pm 34.8)$, $G2 = 444 (\pm 17.5)$, $G3 = 612.8 (\pm 31.2)$. There was a positive correlation between the variables ($p < 0.05$). We conclude that body development of male buffalo is correlated with testicular measurements. Among testicular measurements, SC is the best indicator.

Keywords: male, buffalo, SC, weight.

[Volver](#)

Correlation between alkaline phosphatase of seminal plasma and sperm parameters in buffalos

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The seminal plasma components play a role to modulate the functions and quality of sperm cells. Enzymes as the alkaline phosphatase (AP) have been reported to participate in the spermatozoa metabolism during their epididymal transit. As alkaline phosphatase is produced in high concentrations in the epididymis can be used as a marker to identify the complete ejaculation, oligospermia and azoospermia in many species. Thus, the aim of this study was to assess the correlation between the AP and sperm parameters in buffalos. Eight healthy buffalos were used, with 3 to 5 years-old created in extensive system. The semen samples were collected by electroejaculation and seminal plasma was separated by centrifugation at 900xg/10 min to perform AP analysis. The subjective total motility (0-100%) and sperm concentration in Neubauer chamber were determined. The seminal plasma AP concentration was evaluated by automatic method and (Cobas Mira Plus, Roche Diagnostic Systems and Bioclin Kit, Kinetic, K021, REF Laboratory Quibasa Chemical Basici Ltda). Statistical analysis was performed using Sigma Plot 11.0 software, using Shapiro-Wilk test (normality test) and Spearman correlation. The data was represented by median and quartiles 25% and 75%. The median and quartiles of AP concentrations, the total sperm count and motility were 1,301.0 IU/L (903.5 to 1,851 IU/L), 880 x 10⁶ (687 to 2,350 x 10⁶), 35% (15 to 42.5%) respectively. There was a strong positive correlation between the seminal plasma AP concentrations and total sperm count in the ejaculate ($r = 0.69$, $P = 0.04$) and total sperm motility ($r = 0.70$, $P = 0.04$). We concluded that the AP also produced in epididymis can be represented a complete ejaculation in buffalos.

Keywords: alkaline phosphatase, buffalo, seminal plasma.

[Volver](#)

Application of melatonin implants to induce cyclicity in buffaloes in seasonal anoestrus in northeastern Argentina

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Buffaloes show higher melatonin plasma concentrations during breeding season, in winter, when nights are longer. Continuous-release melatonin implants are used for induction of ovary activity and trigger estrus in spring-summer. The aim of this work was to assess the efficiency of use of melatonin implants in induction of cyclicity in buffaloes in seasonal anoestrus. Twenty adult buffaloes in seasonal anoestrus were used. Ovarian structures were determined by ultrasonography, and blood samples were taken by jugular puncture for Progesterone (P4) dosage. Animals were randomly assigned into one of three treatments (TRT). TRT1 (n=10), on D0 (566.8±76.7pv), buffaloes received melatonin implants (11.2±1.62 implants of 18 mg) and on D9 0.1mg of gonadorelin acetate. TRT2 (n=5), an ovulation synchronization protocol was applied (D0, 2 mg of estradiol benzoate; placement of a P4 intravaginal device until D7, 250.ug cloprostenol; 1mg of estradiol cypionate; D9, 0,1 mg of gonadorelin acetate), and TRT3 (n=5) or control group. Ultrasound monitoring was made, and ovulation was determined by the disappearance of dominant follicle and subsequent corpus luteum formation. P4 concentration on D0 and D17 was established by RIA. Categorical data was analyzed with GENMOD procedure and continuous data with MIXED procedure of SAS. At the beginning of treatments all buffaloes were in anoestrus, they had follicles <8 mm (5.95±1.85 mm) and P4 concentration <1 ng/ml (0.59 ± 0.21 ng/ml). On D10, buffaloes of TRT1 had preovulatory follicle size, while buffaloes of TRT2 and TRT3 had smaller diameter follicles (9.9 vs 6.4 and 6.2mm respectively, p<0.05). At D17, all buffaloes of TRT1 (10/10) presented corpus luteum; 2/5 in TRT2 and 0/5 in TRT3 (p<0.05); P4 levels were higher for TRT1 (4.71 ± 0.53 ng/ml), than TRT2 (1.51±0.75 ng/ml) and TRT3 (0.74±0.75 ng/ml) (p<0.05). Using melatonin implants in buffaloes in seasonal anoestrus induced preovulatory follicle formation, ovulation and subsequent corpus luteum formation.

Keywords: ovaries, spring-summer, progesterone.

[Volver](#)

Effect of administration of equine chorionic gonadotrophin after artificial insemination on pregnancy rates in buffalos in Argentina

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The use of artificial insemination (AI) allow a quick and efficient genetic improvement, in buffaloes the fixed-time artificial insemination (FTAI) is used. High serum Progesterone (P4) concentration during the first three weeks of pregnancies is associated with lower embryonic losses, so use of equine chorionic gonadotrophin (eCG) could benefit the luteal activity because of its luteotropic effect. The aim of this work was to assess the effect of application of 400UI eCG after 14 days of AI on pregnancy rates. FTAI was performed to 645 buffaloes of different categories (138 young buffaloes, 297 buffaloes with calves and 210 dry buffaloes); synchronized by Ovsynch protocol (D0 0,1mg of gonadorelin acetate, D7 0,15mg of D+cloprostenol, D9 0,1mg of gonadorelin acetate); and on D10 insemination was performed. Animals were randomly assigned into one of two treatments (TRT), to receive (TRT1, n=297) or not (TRT2, n=348) 400UI of eCG D14 after FTAI. D14 and D19 after FTAI blood samples were taken by jugular puncture to 120 animals (61 from TRT1 and 59 from TRT2) for P4 plasmatic concentration determination by radioimmunoassay. On D35 after FTAI, pregnancy determination was made by transrectal ultrasonography. Categorical data were analyzed with GENMOD procedure and continuous data with MIXED procedure of SAS. Pregnancy rate was 55,2% (164/297) for TRT1 and 50,3% (175/348) for TRT2, no statistically significant differences were found ($p>0,05$). TRT1 showed the higher pregnancy rates in all categories evaluated, but the differences were not significant ($p>0,05$). P4 concentration on D14 after FTAI were $4,48\pm 3,62$ and $4,28\pm 3,31$ for TRT1 and TRT2 respectively, and on D19 were $3,16\pm 3,22$ and $2,66\pm 2,92$ for the same TRT, no statistically significant differences were found ($p>0,05$). Utilization of eCG after AI in buffaloes was not enough to statistically improve the pregnancy, although the results indicate that further studies on this issue is needed.

Keywords: *corpus luteum*, embryonic mortality, progesterone.

[Volver](#)

Effect of supplementation of *in vitro* maturation medium of buffalo oocytes with essential oil of *Lippia origanoides* on rate embryonic development

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During maturation and culture *in vitro* of embryos, different culture conditions and handling of embryos intensifies the production of reactive oxygen species (ROS), responsible for causing cell damage, leading to embryonic death. The enrichment of medium with antioxidants reducing and preventing damage from free radical. Thus, this study evaluated the effect of supplementation of IVM medium with different concentrations of a natural antioxidant, essential oil of *Lippia origanoides* (EOLO) on the cleavage and buffalo blastocysts rates produced *in vitro* (IVP). We use for maturing 963 buffalo oocytes recovered from ovaries originating from abattoirs, classified as Grade A and B which were divided into 5 treatments: T1 (Medium Base (MB): TCM 199 bicarbonate supplemented with 10% FCS, 22 µg/mL pyruvate, 5 UI/ml LH, 0.5 mg/ml FSH, 1 µg/ml estradiol 83 µg/ml amikacin), T2 (MB + 50 µM/ml cysteamine), T3 (MB + 2,5 µg/ml EOLO), T4 (MB + 5 µg/ml EOLO) and T5 (MB + 10 µg/ml EOLO). Oocytes were matured for 22-24 hours, were fertilized and cultured to the blastocyst stage. The cleavage rate was observed after 48 hours of cultivation and production of blastocysts on 7 and 8. For comparison of means was used ANOVA and Duncan test and the level of significance was $p < 0.05$. The cleavage rates were 39.87 ± 5.54 ; 35.69 ± 6.28 , 44.16 ± 5.68 ; 45.94 ± 6.35 and 43.81 ± 7.58 for the treatments T1, T2, T3, T4 and T5, respectively. No difference was observed between treatments ($p > 0.05$). Regarding the cleavage rate was also no significant difference between treatments T1 (27.45 ± 5.71), T2 (27.92 ± 5.30), T3 (26.51 ± 5.98), T4 (35.05 ± 5.49) and T5 (29.62 ± 6.16) ($p > 0.05$). Despite a significant difference was not noticed, was noted a tendency of increase in blastocyst rate when the IVM medium is supplemented with 5 µg/ml EOLO. Given the results found is possible to say that the enrichment of IVM medium with essential oil of *Lippia origanoides* not compromised cleavage and development of buffalo embryos produced *in vitro*.

Key words: antioxidant, blastocyst rate, buffalo embryo, *in vitro* embryo production.

[Volver](#)

Cell block as a technique for obtaining samples from buffalo testis

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Testicular diseases cause considerable economic losses. The usual biopsy procedure of testis can result in complications that reduce the male capacity for breeding. The cell block technique can be a useful tool in testicular disorders diagnosis because it is minimally invasive method. The aim of this study was to evaluate the cell block technical feasibility of obtaining testicular samples in buffalo. Ten pairs of buffalo testicles were picked at slaughterhouse. The samples were submitted to the cell block technique which consisted of aspiration of testicular tissue with 30x8 hypodermic needle attached to a 10 mL syringe. Subsequently, after removing the needle from the testis 1 ml of 95% ethyl alcohol was aspirated into the syringe. Then completed the total volume of the syringe with a 10% buffered formalin. Macroscopic evaluation was made of each testis. At the same time histological samples of all the testes were taken for comparison purposes. All collected samples were processed by the usual techniques in histology and obtained slides were stained with hematoxylin and eosin technique. The histological slides were examined under a light microscope and the following parameters were evaluated. Presence or not subject to material analysis, quantity of seminiferous tubules intact. After microscopic evaluation 100% of the samples collected by the cell block technique showed suitable material for histologic interpretation. In the sample cell block average seminiferous tubules intact was 40 (22-82). Microscopic examination of the slides with cell material obtained by block addition revealed integrity of the seminiferous tubules, interstitial cells, Sertoli cells and cells of the spermatogenic lineage at various stages of maturation. In comparison with the histological samples was 100% agreement. Based on the methodology used in this study it can be concluded that the cell block technique can be use in obtaining testicular samples in buffalo species.

Keywords: buffalos; testis, cell block; diagnose.

[Volver](#)

Pregnancy rates after repeated transvaginal ultrasound-guided aspirations performed in buffalo cows in Argentina

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The ovum pick up (OPU) is an alternative technique for harvesting high quality buffalo oocytes for in vitro fertilization and in vitro embryo production. Despite of several research reporting that OPU technique is save and repeatable, there are not much information about the effect of repeated puncture above buffalo cow fertility in long term. The aim of this work was to assess if repeated OPU applied in buffalo cows affects pregnancy rates. The trial was carried out on twelve healthy Buffalo cows, and OPU was performed once (n=4) or five times (n=8) during the breeding season. Fixed-time artificial insemination or natural service was performed after the last OPU. Pregnancy diagnosis was performed by ultrasound, and number of days since the last OPU to pregnancy was estimated. Categorical data were analyzed by GENMOD procedure and continuous data by MIXED procedure of SAS. An overall pregnancy rate of 83.3% (10/12) was obtained. The treatment of one OPU showed a pregnancy rate of 100% (4/4), whereas five OPU treatments reach 75% (6/8). No significant differences were found between the two treatments ($p>0.05$). Those buffalo cows that received one OPU conceived 46.25 ± 16.41 , whereas buffalo cows that received five OPU got pregnancy after 66.67 ± 13.4 days after the last OPU. No significant differences were found between the two treatments ($p>0.05$). Oocytes harvesting by OPU technique does not affect pregnancy rate of buffalo cows.

Keywords: Fertility, oocytes, ultrasound.

[Volver](#)

Use of bovine somatotrophin in protocols of artificial insemination in buffaloes

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Artificial insemination has been modified in order to rely less and less heat detection, giving rise to artificial insemination in time fixed (IATF), which consists of a hormonal scheme that allows to perform insemination at the opportune moment to ovulation. Four tests were performed: the year 2012 (E1); 2013 (E2); 2014 (E3) and 2015 (E4); applying the Ovsynch Protocol and the use of somatotropin (bST). 60 buffaloes were used (E1: 32 BST and 28 without bST); 50 buffaloes (E2: 28 BST and 22 without bST); 50 buffaloes (E3: 26 BST and 24 without bST) and buffaloes 62 (E4: 32 BST and 30 non-bST), with a postpartum period between 40 and 45 days and body condition between 3.9 to 4.2 (scale: 1-5); were randomly divided into two groups: bST Group received 500 mg via subcutaneous bST (Lactotropina®, Elanco) five days prior to the first dose of GnRH, another dose at the time of insemination; applied to without bST Group 3 ml of physiological saline, via subcutaneous placebo. The semen used for the four trials was the same; artificial insemination was carried out 18 hours after the last injection of GnRH by the same technical inseminador. 40 days post insemination were diagnosed through rectal palpation and ultrasound examination. The percentage of conception was compared using Chi-square test. The results were significantly different ($P < 0.01$); 56.25 vs 46.42; 64.28 vs 54.54; 59.22 vs 50.78 and 63.42 vs 52.15% of conception for the year 2012; 2013; 2014 and 2015 to E1; E2; E3 and E4 respectively. It is concluded that the application of bST in buffaloes produces a favorable effect on the percentage of conception in IATF protocols.

Key words: buffalo, conception, ovsynch, somatotropin.

[Volver](#)

The use of Doppler ultrasound for evaluating the functional state of the corpus luteum of pregnant and empty Murrah buffaloes

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The Brazilian buffalo herd is estimated at 3.5 million heads, with an annual growth of approximately 3.5%. This shows the great adaptability of this species to the environmental conditions of our country. The objective of this research was to evaluate CL irrigation and perform progesterone levels in empty and pregnant (from the first to the fourth month) Murrah buffaloes. The experiment was conducted at ICA, Mayabeque, Cuba. Ten pregnant buffaloes and ten empty buffaloes were used. The ultrasound device used was a Mindray M5 model with linear transducer of 8 MHz. The CL was evaluated once a month in B-mode and then in Doppler mode. Images were recorded for objective evaluation by software, analyzing the irrigation area of the corpus luteum (COLOR/cm²), the area of the corpus luteum (CL/cm²) and percentage of irrigated area of the corpus luteum in relation to its total area (%). Blood samples were collected by jugular venipuncture and centrifuged at 2500 x g/10 minutes to obtain serum. Serum progesterone concentrations (P4 ng/ml) of the samples were quantified by radioimmunoassay at FMVZ/UNESP Botucatu. ANOVA, Tukey test, Pearson correlation and linear regression were performed for all parameters, assuming p<0.05. To (P4ng/mL) comparing pregnant (5.24±1.62) and empty buffaloes (3.98±1.85), (p<0.05). There was a positive correlation for (COLOR/cm²) and (%) X (P4ng/mL), respectively (r=0.851 and r=0.759 / p<0.001), this not happened for (CL/cm²) X (P4ng/ml) (p=0.075), and linear regression occurred between (COLOR/cm²) and (%) X (P4ng/mL), respectively (R²=72.4 and 57.6 / p<0.001). It was concluded that ultrasound B-mode is not ideal to measure the functional status of the CL, and the use of Doppler mode measuring the irrigation of this area is most appropriate.

Keywords: colour flow, objective evaluation, progesterone, radioimmunoassay.

[Volver](#)

Uterine artery development during pregnancy and its correlation with calves' weight of Murrah buffaloes

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Buffaloes have good adaptability to tropical conditions and are characterized by a good weight growth. They also have an excellent milk quality and palatability of your meat is well accepted. The objective of this research was to evaluate the blood flow of the uterine artery in empty and pregnant (from the first to the fourth month) (E, P1, P2, P3 and P4) Murrah buffaloes and its correlation with calves weight at birth. The experiment was conducted at ICA, Mayabeque, Cuba. Ten pregnant buffaloes and ten empty buffaloes were used. The ultrasound device used was a Mindray M5 model with linear transducer of 8 MHz. The uterine arteries were located and its diameter was measured. Resistive index (RI), pulsatility index (PI) and time average medium velocity (TAMV) were measured by Doppler mode. Blood volume (ml/min) (VOLUME) was calculated using Bollwein et al. (2002) equation: $VOLUME = TAMV \times \pi \times (D \times (0.1/2))^2 \times 60$. After birth calves were weighed (WEIGHT). ANOVA, Tukey test, equivalence test and Pearson correlation were performed for all parameters, assuming $p < 0.05$. Comparing the groups E, P1, P2, P3 and P4, $p = 0.009$ for the uterine arteries diameter average (cm) (UA), respectively (1.134^B, 1.290^{A,B}, 1.59^A, 1.55^A and 1.44^{AB}), and $p = 0.037$ for VOLUME, respectively (117^A, 159^{AB}, 229.5^A, 152.2^{AB} and 190^{AB}). Comparing studied parameters between ipsilateral and contralateral to the pregnancy in the paired equivalence test for P1, P2, P3 and P4, it can be stated that UA ipsilateral is greater than UA contralateral on the P1 ($p = 0.026$). There was a positive correlation between VOLUME and WEIGHT ($r = 0.943$ / $p < 0.001$). It was concluded that during the pregnancy occurs an increase in the diameter and in the blood volume of the uterine arteries, both as ipsilateral as contralateral to the pregnancy, and it is highly correlated to the weight of the calves at birth.

Keywords: Doppler ultrasound, spectral, blood volume.

[Volver](#)

Morphologic evaluation of testicular and epididymal structures from buffaloes before puberty by magnetic resonance

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The aim of this study was to report the main anatomic aspects of testis and epididymis from buffaloes, using magnetic resonance (MR), as well as their relation with age and body weight. Ten pairs of testis and epididymis from healthy buffaloes with an average age of 20.2 months obtained at a local slaughterhouse were used. The collected material was maintained at -20°C until being analyzed. An Esaote® Vet MR Large with 0.25 Tesla of magnetic field and four radiofrequency channels was used for MR evaluation. Sections of 5.0 mm were performed in sequence: 3.0 mm of Spin eco T1 transversal, 18.0 cm FOV; 3.0 mm Spin eco T2 sagital, 18.0 cm FOV; 0.6 mm Sagital Turbo 3D T1; 3.0 mm thickness STIR sagital, 18,0cm FOV; 3.0 mm Gradiente eco T2 Sagital, 10.0cm FOV; 4.0 mm Spin eco T2 Dorsal section, 16.0 cm FOV. Testicular parenchyma (TP); mediastinum (MD); head (EH), body (EB) and epididymis tail (ET) were evaluated. Images were evaluated by Synapse® software, considering measurements of testicular parenchyma (TP), mediastinum (MD), epididymis head, body and tail represented as: EH, EB and ET, respectively. Results were expressed according to the degree of similarity. The images evaluated for age and weight had TP results with 71.07% similarity with GE T1, 64.50% in STIR and 59.21% with the other measurements (GE T2 and T2 FSE). MD similarity level with GE T2, FSE T2 and STIR images was 60.10% and 40.65% at GE T1. EB and EB had 64.51% of similarity with GE T1, FSE T2 and STIR; and 59.21% with GE T2. On ET similarity level was the same for all images (45.55%). The obtained results confer tissue difference of analyzed structures by MR, regarding age and weight of the animal, providing predictive morphological data for this species at this age group characterized by puberty.

Keywords: buffalo, puberty, reproductive morphology, veterinary imaging.

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Descriptive evaluation of testis and epididymis from cattle and buffalo by magnetic resonance

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The aim of this study was to characterize morphological particularities and occasional anatomic variations of testicular and epididymal structures from buffalo and cattle by magnetic resonance (MR), as well as evaluate the applicability of this technique. Five pairs of testis and epididymis of clinically healthy animals were used, with an average age of 18.5 years and weight of 550.15 kilograms slaughtered at a local slaughterhouse. After collection the material was stored at -20°C until being analyzed. An Esaote® Vet MR Large with 0.25 Tesla of magnetic field and four radiofrequency channels was used for MR evaluation. Sections of 5.0 mm were performed in sequence: 3.0mm of Spin ecoT1 transversal, 18.0 cm FOV; 3.0 mm Spin ecoT2 sagital, 18.0 cm FOV; 0.6 mm Sagital Turbo 3D T1; 3.0 mm thickness STIR sagital, 18.0 cm FOV; 3.0 mm Gradiente ecoT2 Sagital, 10.0 cm FOV; 4.0 mm Spin ecoT2 Dorsal section, 16.0 cm FOV. Testicular parenchyma (TP); mediastinum (MD); head (EH), body (EB) and epididymis tail (ET) were evaluated. The images were analyzed using Synapse® software. Results were expressed according to the degree of similarity. The PT images from cattle and buffalo showed 100% similarity level in GE T1 and STIR, followed by 33.33% for the others (GE, T2, FSE T2). Regarding MD, the images that most closely resembles were GE T2 and T2 FSE (87.3%), whereas GE T1 and STIR exhibited 62.5% similarity and 37.5%, respectively. For epididymis EH and EB, level of similarity between species was higher in FSE T2 and STIR (100%), and lowest for GE T1/ T2 (41.70%). For ET images, only GE T1 was 100% similar for both species. For the other images the similarity level was 58.3%. PT and MD data were attributed to structural difference of tissues. Epididymis differences observed between species relates to fluid concentration and adipose tissue. It was concluded that the RM. have great applicability for morphological characterization of cattle and buffalo testicles.

Keywords: males, reproductive tract, veterinary imaging.

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Thermographic evaluation of breast development crossbred murrh buffaloes during late pregnancy

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The aim was to evaluate thermal parameters of the mammary gland from buffaloes in late pregnancy. Six crossbred females Murrah, with mean age of 21 months and weight 490.7kg, raised in an extensive management, were evaluated each 28 days, during the last four months of pregnancy. Digital thermography infrared tests were carried out of the mammary gland by using a thermographic camera (Flyn E40) and the images were analyzed by Flir Tools[®] program. The surface temperature (°C) of the mammary glands cisterna; base, middle third and extremity of the teat from previous right and left mammary quarters and side view from later right and left mammary quarters. Rectal temperature (°C) was also measured. Analysis of variance and Tukey 5% were used. There was statistical difference only among evaluated month, while being the third month of evaluation the most significant. Rectal temperature recorded change was 37.0 to 37.9 °C. For mammary glands cistern, base, middle third and extremity of the teat from the previous right and left mammary quarters; and later left and right, the recorded change was 21.6 to 37.6 °C. Therefore thermography detected changes in breast surface temperature, especially in late pregnancy, where the breast development is more intense, reflecting physiological changes expected for this phase.

Keywords: tomographic parameters, dairy buffalo, mammogenesis.

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Synchronization and resynchronization of oestrus and fixed-time artificial insemination in buffaloes in Argentina

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Resynchronization programs are useful when artificial insemination (AI) needs to be carried out in the largest number of animals in a short period of time, and allows the return to oestrus of those buffaloes that were not pregnant at fixed-time artificial insemination (FTAI). This technique is able to increase the number of calves born, increasing the farm productivity. The aim of this work was to assess the implementation of synchronization and resynchronization of oestrus in a herd of buffalo in Argentina. Two hundred buffaloes from different categories (buffalo heifers n=55; lactating buffalo cows n=98; and dry buffalo cows n=47) were synchronized for FTAI by Ovsynch protocol. Resynchronization protocol began at 20 days after FTAI, an ultrasound diagnoses were performed 27 days after FTAI, and then resynchronization protocol continued on the 66 non-pregnant buffaloes (buffalo heifers n=32; lactating buffalo cows n=17; and dry buffalo cows n=17). At 30 days after the last AI a second ultrasound diagnoses were performed to evaluate pregnancy rate of resynchronization protocol. Categorical data were analyzed by GENMOD procedure and continuous data by MIXED procedure of SAS. Pregnancy rate on first AI was 49% (98/200), and 42.2% (28/66) for the second one. An overall pregnancy rate of 63% (126/200) was obtained in an interval of 30 days. Pregnancy rates in buffalo heifers were 58.2% (32/55) and 34.4% (11/32) for first and second AI respectively. Significant differences were found ($p < 0.05$); whereas in lactating buffalo cows pregnancy rates were 43.9% (43/98) and 47.1% (8/17), and in dry buffalo cows reach 48.9% (23/47) and 52.9% (9/17) for first and second AI respectively, no significant differences were found in these case ($p > 0.05$). Pregnancy rate obtained in this work shows that the application of resynchronization program in buffalo is useful to increase the herd productivity using a superior buffalo bull.

Keywords: insemination, Ovsynch, ultrasound.

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Buffalo population growth in Argentina

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First buffaloes arrived to Argentina at the beginning of twentieth century. Many attempts were carried out to get hybrids between beef cattle and buffalo, however those attempts failed because chromosomal differences. Buffalo production took up again at the beginning of the 1980s. There is a wide underexploited region on the north of Argentina that can reach about eight million hectares. This area has a stocking rate of one buffalo every two hectares. The aim of this work was to assess the growth of buffalo population in the last years in Argentina. Data from the National Service of Animal Health and Agriculture and Food Quality (SENASA for its acronym in spanish) from different provinces of Argentina during the years 2014 and 2016 were used. Seven categories were taken into account and percentage difference between each one, on both years, were evaluated. Continuous data were analyzed with MIXED procedure of SAS. Buffaloes inhabit 21 provinces out of the 23 that integrate the national territory. There are not Buffaloes existence in Chubut and Tierra del Fuego, the coldest provinces of Argentina. Considering the division of the country by region, the northeast region of Argentine (NEA), composed by Formosa, Corrientes, Chaco and Misiones provinces, concentrates 85% of the total Buffalo population. The percentage of contribution from the different categories to the total Buffalo population is as follow: buffalo cow (45.55%); buffalo heifer (14.64%); female buffalo calf (10.98%); male buffalo calf (11.63%); young buffalo steer (13.27 %); and buffalo bull (3.93%). Buffalo population in July 2014 was 87711 head, whereas in March 2016 the population reached 101555. These finding represent an annual growth of 9.47%. Buffalo production in the country and mainly in the NEA is in true expansion and presents all conditions and competitive advantage to become a successful production system.

Keywords: categories, population, northeast region of Argentina.

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Scrotum and neck digital infrared thermography and semen quality of Nelore bulls and Murrah buffaloes bulls

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To optimize the reproductive function for natural mating or artificial insemination, bulls should produce a large number of morphologically normal sperm. A moderate increase in testicular temperature of the bulls submitted to scrotum insulation dramatically reduces semen quality. The objective was to study the relationship between scrotal surface temperature and semen quality in nine Nelore bulls and nine Murrah buffaloes bull, raised in extensive system. Were performed scrotal and neck thermography with Flir E40[®] camera of pre and post seminal harvest and held the semen by electroejaculation. The thermograms were analyzed with Flir Tools[®] software for surface scrotal temperatures, right and left sides, making the average between the two for statistical evaluation, proximal and distal. ANOVA, Tukey test and Pearson correlation were performed, assuming $p < 0.05$. Nelore bulls had lower preharvest neck temperature than buffalo bulls, indicating lower management stress ($p = 0.006$), (29.2°C and 26.789°C). Buffaloes bulls had lower semen quality than bulls ($p < 0.05$). Respectively for volume, motility, vigor and mass movement (5.22mL and 3.056mL, 71.11% and 29.44%, 2.66 and 1.33, 2.44 and 1.22). Correlating the thermographic data with sperm analysis data of buffaloes bulls was a positive correlation between preharvest proximal testicular temperature X preharvest distal testicular temperature ($r = 0.688$ / $p = 0.040$), postharvest proximal testicular temperature X preharvest proximal testicular temperature ($r = 0.785$ / $p = 0.012$) postharvest neck temperature X postharvest proximal testicular temperature ($r = 0.763$ / $p = 0.017$), volume X postharvest distal testicular temperature ($r = 0.838$ / $p = 0.005$) and postharvest neck temperature X volume ($r = 0.679$ / $p = 0.044$). It was concluded that Nelore bulls have better seminal quality than Murrah bulls, there was interrelationship between the proximal and distal testicular temperatures, the proximal testicular temperature is more susceptible to changes in the neck temperature and scrotal surface temperatures influence the quality of buffalo semen.

Keywords: stress, testis, spermatozoa.

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The Relationship between the Concentrations of Anti-Mullerian Hormone, Type of Follicle Stimulating Hormone and Ovulatory Response to Superovulation in Water Buffaloes

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The widespread use of improved water buffalo genetics has been limited as a result of variable and poor yields of embryos following superovulation treatments. The objective of this study was to determine the relationship between circulating concentration of anti-mullerian hormone (AMH) and type of FSH preparation on follicle growth and ovulatory response in water buffaloes. Two blood samples were collected from female water buffaloes (N=31; age: 9.06 ± 0.98 years; age range 1.5 to 22.3 years) 6 months apart and analyzed to determine systemic concentration of AMH. Animals with concentrations of AMH higher or lower than 194 pg/ml were placed in the HIGH and LOW AMH groups and were randomly assigned to be superovulated with either FSHp (high LH content) or Follitropin (low LH content). Follicular growth and ovulation was monitored using transrectal ultrasonography. Analysis of variance was conducted to analyze the fixed effects of AMH concentration, type of FSH and their interaction on number of small, medium and large follicles and number of follicles ovulating. The within female repeatability of concentration of AMH was 0.97. Concentration of AMH varied quadratically with age ($P < 0.001$). FSHp treated females had a higher number of 3-5mm follicles than Follitropin treated animals ($P = 0.0028$: 1.59 ± 0.19 vs 0.74 ± 0.17 , respectively). Follitropin treated females had a higher number of > 10mm follicles ($P < 0.001$: 4.55 ± 0.36 vs. 1.54 ± 0.40 , respectively) and number of disappearing follicles ($P = 0.001$: 6.3 ± 0.7 vs. 2.56 ± 0.78) than FSHp females. Females in the HIGH AMH group tended to have a higher number of disappearing follicles ($P = 0.06$: 5.44 ± 0.78 vs. 3.42 ± 0.70). In conclusion selecting animals with high AMH concentration and the use of FSH preparations with a lower LH content can be used to improve the superovulatory response in water buffaloes.

Keywords: assisted reproductive technology, follicle stimulating hormone, ovarian reserve, superovulatory response, water buffaloes.

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Polymorphisms in TLR4 gene and their association to milk production traits in buffaloes

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Mastitis is a disease that negatively influences milk production and quality, affecting the dairy industry efficiency. Molecular markers might be developed to investigate genetic variants associated to the disease and assist selection process in order to identify resistant animals. When the mammary gland is infected, there is an increase in the defense cells, also called somatic cells. It is a defense line of the immune system against pathogens. The "tool like receptors" TLR are membrane proteins that have an important role in the immunity, recognizing pathogens and activating adequate responses (Ruiz *et al.*, 2011). The present study aimed to investigate the association of TLR4 gene SNPs. With the somatic cell counting (SCC) in buffaloes. The DNA was extracted from hair follicles of 160 Murrah buffaloes. The fragments were amplified by Polymerase Chain Reaction (PCR) and sequenced. Thirteen SNPs were found. Allelic and genotypic frequencies were calculated as well as the adhesion to Hardy-Weinberg equilibrium and the linkage disequilibrium (r^2) and the association to the somatic cell counting (SCC). Was tested methodology linear generalized mixed model, assuming Poisson distribution. Bonferroni correction was applied for the number of SNPs. Thirteen SNP polymorphisms were identified in coding region of the TLR4 (g322>G/A, g514>C/T, g536>A/T, g8338>A/C, g8341>A/G, g8342>T/G, g8343>G/A, g8345>A/G, g8413>A/G, g8428>G/A, g8438>A/C, g8578>G/T, g8582>A/C). All the SNPs were associated ($p=0.001$). Other authors also reported the association of TLR4 SNPs to the trait. The results show that the SNPs of TLR4 gene can be used as molecular markers in buffaloes.

Keywords: molecular markers, Snp, somatic cells.

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Exogenous control of ovulation in buffalo heifers during unfavorable reproductive season in tropical region

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The buffalo species has a reproductive seasonality which compromises the distribution of milk production throughout the year interfering in the market, with drastic fluctuations in the prices of dairy products. The induction of ovulation in heifers during unfavorable breeding season by hormone therapy and TAI is an alternative to make stable milk production over the year favoring the consumer market. It was aimed to evaluate three ovulation-inducing hormones in fixed time artificial insemination protocol. Twenty four buffalo heifers were used, divided into G1 (n = 8; 21.36 ± 0.7 months and 338.26 ± 14.40 Kg); G2 (n = 8; 22.16 ± 2.12 months and 335.00 ± 24.04 kg) and G3 (n = 8; 21.05 ± 0.0 months and 338.66 ± 31.81 kg). In TAI protocols it was used GnRH, progesterone, prostaglandin and eCG. The groups differed in the method used for inducing ovulation, where G1 received only 0.25 mg of gonadorelin acetate, G2, 0.25 mg gonadorelin acetate followed by 0.75 mg of D-cloprostenol, and G3, 0.150 mg of d-cloprostenol as inducing agent. The data of follicular dynamics were analyzed by ANOVA. There were no statistical differences between groups regarding the growth rate of the dominant follicle, the diameter of the preovulatory follicle, the corpus luteum diameter, intervals between withdrawal of progesterone device and ovulation and ovulation rate which was 87.5 % for the three groups. It was concluded that the induction of ovulation during unfavorable breeding season is possible with satisfactory results, as well as it was evidenced the ability of D-cloprostenol to induce ovulation in buffalo heifers, associated or not to GnRH, and that the association of GnRH and D-cloprostenol did not maximizing the ovulation rate.

Keywords: female buffalos, artificial insemination, estrus, follicular characteristics.

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Expression of prostaglandin F2 α , oxytocin and oestrogen receptors in endometrial biopsy samples throughout the puerperium in buffaloes treated with cloprostenol in the third week postpartum

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The present study investigated expression of prostaglandin F2 α (PGF2 α), oxytocin and estrogen receptors in uterine tissues of postpartum buffaloes treated with cloprostenol in the third week postpartum. Twenty clinically healthy postpartum adult Murrah females were treated with saline solution (treatment CONT) or cloprostenol (treatment CLO15) administered 15 and 20 days postpartum. All cows included in the study had a normal delivery without retained placenta. Endometrial biopsy was carried out on days 2, 7, 14, 21 and 28 postpartum to quantify *PTGFR*, *OXTR* and *ESR1* expression, using a Yomann biopsy nipper. The endometrial samples were washed in phosphate-buffered saline, put into micro tubes containing RNA later[®], kept at 50C for 24 h and then stored at -80°C. Endometrial samples were macerated and total RNA extraction was performed using the RNEasy Mini Kit, according to manufacturer's instructions, and treated with DNase to prevent DNA contamination. The RNA was quantified in a spectrophotometer. Reverse transcription was performed using the commercial Superscript III First-Strand Synthesis Supermix kit, according to the manufacturer's specifications. Real time PCR was performed using the Power SYBR Green PCR Master Mix kit, according to the manufacturer's instructions. Means of relative gene expression were contrasted by Student-Newman-Keuls test. Differences were considered significant at the 95% confidence level ($P < 0.05$). *OXTR*, *PTGFR* and *ESR1* expression was similar between the treatments and the days of evaluation ($P > 0.05$). In conclusion, cloprostenol administration at 15 and 20 days postpartum seems to have no influence in these receptors expression.

Keywords: *PTGFR*, *ESR1*, *OXTR*, RT-PCR, *Bubalus bubalis*.

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Pregnancy rates of buffaloes (*Bubalus bubalis*) using cooled or frozen semen at fixed time artificial insemination: preliminary results

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The objective of the study was to compare the fertility of cooled (5°C/24 hours) versus frozen semen for FTAI in cycling buffaloes submitted to Ovsynch protocol. A passive system of cooling was used (refrigerator/average - 0.25 °C/min.) and semen was diluted in a commercial extender (BotuBov® BotuPharma®, Botucatu/SP, Brazil) for cooling and freezing. The experiment was conducted at 20° 41' 45" South latitude and 44° 49' 37" West longitude, in May and June 2016. Eighty five females, aged 3-17 years; BSC = 4 (1-5); weighing 500 to 720 kg were randomly divided into three groups: G1 (n = 35), G2 (n = 30) and G3 (n = 25), each one synchronized and inseminated in three different periods. The ejaculates of each bulls (n = 2) were split into two aliquots (one for cooling and the other for freezing) and diluted to a final concentration of 50x10⁶ SPTZ/mL. Protocol consisted of: injection of 2 mL i.m. GnRH (Cystorelin®, Merial, USA) on D0 (14:00hs), 4 mL i.m. of PGF_{2a} (Croniben®, Biogenesis - Bagó, BA, Argentina) on D7 (14:00hs) and 2 mL i.m. GnRH (Cystorelin®) on D9 (14:00hs) AI (~ 20x10⁶ motile SPTZ) was performed on D10 (beginning at 6:00hs am). Each type of semen was alternately used in the cows of each group. Pregnancy diagnosis was performed by ultrasonography (Aloka SSD-500, 5 MHz probe, Tokyo, Japan) on D30. Total conception rates were compared by the Fisher's exact test. Overall conception rates of 54.5%^a (24/44) and 31.7%^b (13/41) (p<0.05) for cooled and frozen semen, respectively. In conclusion, the use of cooled semen is a promising option to increase the conception rate at FTAI of buffaloes.

Keywords: buffaloes, cooled frozen semen, pregnancy rate, FTAI.

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Prospecting genome regions related to wither height in buffalo

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Genome Wide Association Study (GWAS) is an efficient method to discover QTL affecting phenotypes. The aim of this study was assess the association of genomic information with a linear trait in water buffaloes, by performance of ssGWAS, and prospect genes underlying this trait. A total of 322 Murrah cows genotyped with the Axiom® Buffalo Genotyping Array, which contains around 90K single nucleotide polymorphism (SNP), were used to perform a GWAS using wither height (WH) phenotypes. The quality control procedures were performed with the Plink v 1.7 software, and were filtered out all markers with call rate lower than 0.95 and MAF lower than 0.05. SNPs that departed from Hardy-Weinberg equilibrium ($P > 10^{-6}$) or had no autosomal location were also excluded. All samples presented call rate higher 0.90, than they were all left to analyses. The total number of animals with phenotypes was 674. The ssGWAS is based on single-step methodology where the pedigree and genomics information were combined in the H matrix. After detecting association, the presence of genes over those genomic region were investigated according with the cattle genomic coordinates of UMD3.1. Around 45K SNPs passed for quality control procedures. We found three significant regions related with WH phenotypes, located on BBU1, BBU3 and BBU23 that corresponds to BTAs 27/21/1, 8 and 26, respectively, following alignment proposed previously by our group. There were 29 genes in these regions, which are mainly related to metabolic pathways of the cells growth and immune system. A noteworthy gene detected in this study was the *Csmd1*, which is related to weight gain and body growth in mice, than corroborates the results of the current work. This study suggests that *Csmd1* gene is related to bone and muscle growth and may explain the variability of WH in buffalo.

Keywords: *Csmd1*, GWAS, Linear traits.

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Expression profile of Toll-like receptor 2 gene in dairy buffaloes with mastitis

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Toll-like receptors (TLRs) are a group of protein essential for the detection of pathogens and the initiation of immune response. Thus, the aim of this study was to evaluate the relative expression of Toll-like receptor 2 (TLR-2) gene in milk cells of dairy buffaloes with mastitis. Milk samples of 10 animals with mastitis and 10 animals without mastitis was used for RNA extraction by Trizol[®] reagent (Invitrogen, USA). The verification of mastitis was performed by California Mastitis Test and somatic cell count. The RNA quality and concentration were measured by spectrophotometer and the absence of contamination by genomic DNA was confirmed in a Qubit[®] 2.0 fluorometer (Invitrogen, USA). The cDNA was amplified by quantitative real-time PCR using specific primers. RPLP0 primer were obtained in the literature, while TLR-2 and EEF1A1 primers were described on the literature to bovine studies and redesigned from the homologous buffalo mRNA. The EEF1A1 and RPLP0 genes were tested by the geNorm program and used as endogenous controls for normalization of TLR-2 gene expression. Data were analyzed using the PROC MIXED of Statistical Analysis System (SAS Institute Inc., Cary, NC). The $\Delta\Delta C_t$ was estimated using orthogonal contrasts between treatments and endogenous controls and target genes. Values were considered significant when the approximation of $\Delta\Delta C_t$ showed $p < 0.05$. The TLR-2 gene presented a significant difference ($p < 0.0001$) between the groups of animals with and without mastitis. The relative expression of TLR-2 gene was 13.34 times greater in buffaloes with mastitis. This result may provide benefits and high impact on mastitis control in dairy buffaloes, because this gene can be considered a candidate gene associated with resistance to this disease.

Acknowledgements: The authors thanks (Grant n° 2014/19321-4 and 2014/25309-7) São Paulo Research Foundation (FAPESP) (Brazil) for financial support of this research project.

Keywords: *Bubalus bubalis*, gene expression, quantitative real-time PCR, TLR-2.

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Buffalo embryo production using ionomycin

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The parthenogenetic activation may be used as a device to test the quality of cultivation, this activation is achieved through the use of substances such as ionomycin to increase stimuli that mimic the fertilization process, providing calcium signals to the oocyte by different mechanisms. The objective of this study was to induce parthenogenetic activation using ionomycin followed by an immediate exposure to 6-DMAP, and to observe embryonic development of buffalo oocytes to the blastocyst stage at day 7 (D7) and, indirectly, to assess the quality and competence of oocytes after maturation. The buffalo ovaries were used to obtain COCs after having been washed oocytes were denuded with the help of hyaluronidase, following washing in SOF, and were put five minutes in contact with ionomycin, and then washed again in SOF and went to 6-DMAP treatment where they were kept for 4 hours. After the time structures were again washed in SOF and followed for culture where they remained for 7 days. Embryos were evaluated on day 3 to verify cleavage rate and on day 7 to assess blastocyst rate. Cleavage rate was 68% (17/25) and blastocyst rate was 36% (9/25). The co-culture of denuded oocytes with cumulus cells increases their meiotic competence which was used in the experiment after parthenogenetic activation, possibly contributed to the results achieved. The method used in this study to induce activation resulted in a good rate of cleavage and embryonic development probably because successfully mimics the events of fertilization. In conclusion, we have demonstrated the possibility of chemical activation of buffalo oocytes in vitro with ionomycin, followed by immediate exposure to 6-DMAP. And that growing conditions can be identical to those used in cattle.

Keywords: buffalo, oocytes, parthenogenetic activation.

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Efficiency of genotype imputation in buffalo's metacentric chromosomes rearranged from bovine reference

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The assembly of a reference genome is essential for genome-wide studies, which requires the exact position of SNPs in any species. Buffalo specie has only scaffolds and annotated genes available, but the ordination of they are still not assembled. Previous studies carried out by our group observed the possibility of rearranging the buffalo's chromosomes using bovine reference map, by linkage disequilibrium between chromosomal structures. Thus, the aim of this study was to evaluate the imputation efficiency on five buffalo's chromosomes with (R_BBU) and without (NR_BBU) rearrangement. A total of 352 buffaloes were genotyped with 90K Axiom Buffalo Genotyping (Affymetrix). A total of 46,378 SNPs passed in the genotype quality control (call rate ≥ 0.95 , MAF ≥ 0.05 , P-HWE $< 10^{-6}$). Only autosomal markers with known position (UMD3.1) were used. The software Beagle v.4 was used for genotype imputation. Scenarios that considered 10%, 15%, 20%, 25%, and 30% of the markers were used to calculate the allelic concordance rate (CR) and allelic R-square (r^2). The analyses were performed dividing the animals into reference and 88 imputation sets in a 4-fold cross-validation scheme. Considering full chromosomes, the average of accuracy was similar between R_BBU and NR_BBU but varied between scenarios, ranging from 0.87 to 0.91 (r^2) and 0.92 to 0.96 (CR). Only for R_BBU1 (scenario 10%) and R_BBU4 (scenario 30%) a significant increase ($P < 0.05$, t-test) in the CR was observed. However, considering one Megabase window to either side of the centromere, we found a more evident difference in the imputation accuracy for all five chromosomes in most scenarios. An increase of 11% and 9% in the r^2 and CR, respectively, was observed on R_BBU1. Therefore, the rearrangement was appropriate for increase imputation efficiency due to a better construction of haplotypes.

Key words: genomic selection, Murrah, genomic assembly.

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Selection signatures scan in water buffaloes

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In contrast to demographic factors, the positive selection influences only genomic regions related to selected phenotypes, leaving special patterns of DNA called selection signatures. The statistics "Runs of Homozygosity (ROH) enable the identification of parts of genome presenting reduced local diversity due selection. The current study aimed to detect selection signatures in the water buffaloes genome, by scanning ROH hotspots. A sample of 352 Murrah buffaloes, explored to milk production in Brazil, were genotyped with Axiom® Buffalo Genotyping Array (Affymetrix). This array is based coordinates of cattle genome reference (UMD3.1), however, our group previously perform a LD based alignment to predict buffalo genome position to each marker, enabling this first selection signature scan in this specie. A total of 55,560 autosomal markers with predicted position to buffalo genome and presenting call-rate>0.95 were used to the ROH scan, with the PLINK v.1.7 software. The uninterrupted sequences with more than 30 homozygous SNPs were assumed ROHs. Then, a local autozygosity coefficient was estimated to each marker, as the proportion of individuals presenting a ROH enclosing the marker, being values higher than 0.3 assumed selection signals. Gene and QTL within selection signatures were retrieved from *Ensembl* e *CattleQTL* databases, based on bovine homologue regions. Four genomic regions were ROH hotspots, located at BBU3 (60094330-65022593), BBU4 (43407621-44282612), BBU5 (57022610-60442072) e BBU16 (46166532-50868968). Based on bovine information, there were 202 annotated genes e 631 known QTL over these selection signatures. The *ILK* gene that has a central role in hair follicle development and skin thickness, is a notable gene in this list, specially, because buffaloes are markedly represented by animals of low hair density and thick skin. In addition, this first study of selection signatures in buffaloes underscore the utility of this practice to evidence signals of past selection and genetics regions underlying important traits.

Keywords: autozygosity, *Bubalus bubalis*, Murrah, runs of homozygosity.

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Comparison of pregnancy rate in water buffalo (*Bubalus bubalis*) after ovulation synchronization and timed artificial insemination using two different progesterone intra-vaginal devices

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Timed artificial insemination (TAI) programs in water buffalo have adopted hormonal protocols used in cattle. However, physiological differences (e.g. progesterone levels) may affect the efficacy of the synchronization programs in this species. The objective of this study was to compare the pregnancy rate after ovulation synchronization and TAI in water buffalo (*Bubalus bubalis*) using two different intravaginal progesterone (P4) devices containing different (P4) concentration. The study was performed at a commercial buffalo farm at Colón county of Zulia state, Venezuela, during June and August 2015. One hundred six mature female buffalo were utilized in this study. Animals were randomly assigned to one of two groups that received an intravaginal device for 9 days: 1) CIDR (n= 55) (CIDR®, Pfizer) containing 1.38 gr of P4 or 2) DIB (n= 51) (DIB®, Syntex) containing 1.0 gr of P4. Additionally, on day 0, animals were administered a dose of 50 µg of GnRH (Conceptal®, Intervet) im. On day 9, the intravaginal devices were removed and animals received 25 mg de PGF2α im (Lutalyse®, Pfizer) and 500 UI of eCG (Folligon®, Intervet) im. On day 11, animals received 50 µg of GnRH (Conceptal®) im and were artificially inseminated 8-12 hours later, using semen from a single Murrah bull. Animals had a body condition score of 3 and 3.5 (scale 1 to 5). Data were analysed using proc logistic of the Statistical Analysis System (SAS). Animals in the DIB group had a significantly higher pregnancy rate (62.7%; 32/51; $P = 0.0193$) compare to animals in the CIDR group (40%; 22/55). In conclusion, pregnancy rate was higher in buffaloes treated with the synchronization protocol using DIB compared to that obtained with CIDR, which might be attributed to differences in P4 concentration, chemical composition or absorption rate.

Keywords: water buffalo, timed artificial insemination (TAI).

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Efecto de la hormona liberadora de gonadotropinas y la gonadotropina coriónica humana como inductores de ovulación en IATF en búfalas (*Bubalus bubalis*)

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La inseminación artificial es una alternativa para favorecer el incremento genético en búfalos. El uso de programas de inseminación artificial a tiempo fijo (IATF), ayuda la sincronización de los ciclos estrales y ovulaciones. A nivel nacional con IATF en búfalas reportan tasas de preñez de 26,8% (Serna *et al.*, 2015), y promedio nacional del 30%; con un rango del 5-70% (Carvalho et al 2013). El objetivo del trabajo fue evaluar el efecto de GnRH y hCG como inductores de ovulación en programas de IATF, comparando las tasas de preñez. La investigación se realizó en el municipio de Rionegro (Santander- Colombia) con una temperatura media de 25 °C. Se usaron 50 búfalos de la raza Mediterránea, con 30 a 45 días postparto, de 3 a 4 partos y se dividieron en 5 tratamientos con 10 animales, manejando un diseño completamente aleatorio. Los animales fueron sometidos a IATF, con dispositivo intravaginal de progesterona de 1 gr y distribuyeron así: Tratamiento I: GnRH (12 horas antes del servicio), tratamiento II: GnRH (al servicio), tratamiento III: hCG (al servicio), tratamiento IV: GnRH (12h antes servicio) + hCG (al servicio), tratamiento V: Control. La inseminación artificial se realizó con semen congelado de un mismo toro. Finalmente se realizó un ANOVA para analizar los resultados. Se obtuvo para el TI un 60% de tasa de preñez (6/10), para el tratamiento II y TIII un 40% (4/10), respectivamente, para el tratamiento TIV un 50% (5/10) y para el TV un 30% (3/10), encontrándose diferencias estadísticamente significativas entre el tratamiento I y el resto de los tratamiento analizados. Se concluye que el uso de la GnRH 12 horas antes del servicio genera mejores tasas de preñez que la HCG, favoreciendo la fertilidad en búfalos.

Keywords: biotecnología reproductiva, fertilidad, tasas de concepción, sincronización de estros.

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Identification of sperm subpopulations with motility sperm patterns in frozen-thawed Murrah buffalo semen

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The aims of this study were to identify the existence of sperm subpopulations (SP) with specific motility characteristics in frozen-thawed ejaculates of five Murrah buffalo bulls, by using a computer-assisted sperm motility analysis (CASA) system, and to establish the existence of between-bull variation in the SP structure. Data from 11,877 motile spermatozoa, defined by curvilinear velocity (VCL), linear velocity (VSL), average path velocity (VAP), linearity coefficient (LIN), straightness coefficient (STR), wobble coefficient (WOB), mean amplitude of lateral head displacement (ALH) and frequency of head displacement (BCF) were analyzed using a multivariate clustering and chi-squared procedures to identify and quantify these spermatozoa into a reduced number of SP according to their movement patterns and frequency of SP by bull. The statistical analysis clustered all the motile spermatozoa into four separate SP with defined patterns of movement: SP1 (25.57%), characterized by linear sperm highly active (LIN= 68.73%, STR= 83.66%), but slow velocity (VAP=33.10 $\mu\text{m/s}$); SP2 (38.15%), includes poorly motile (VAP=14.82 $\mu\text{m/s}$) and non-linear spermatozoa (LIN=27.91%); SP3 (15.84%), represented by rapid, progressive and highly active spermatozoa (VAP=77.38, VCL=89.32 $\mu\text{m/s}$, LIN=79.55% and STR=90.32%); SP4 (20.44%) represented by spermatozoa with moderate velocity (VAP=49.54, VCL=82.38 $\mu\text{m/s}$), high ALH (4,03 μm) but non-linear spermatozoa (LIN=31.04% and STR=51.37%). There were significant differences in the distribution of the four SP ($P<0.001$). The frequency distribution of spermatozoa within SP were different for the buffalo bulls evaluated ($P<0.001$), but the higher differences were mainly in SP2 (29.70 to 54.08%). Four well-defined motile SP were identified. Our results show that the use of the CASA system is a relatively simple approach to the study of SP patterns, and could lead to a substantial increase in information acquired during semen analysis in buffalo bull ejaculate.

Keywords. CASA system, cryopreservation, movement, spermatozoa.

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Evaluation of the sperm acrosome integrity in cryopreserved buffalo semen using two staining methods: sperm-blue® and eosin-nigrosin

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Semen cryopreservation result in sublethal damage on integrity sperm membrane, increasing the number of spermatozoa with acrosome reaction affecting the fertilization process. Eosin-Nigrosin (EN) staining have been proven effective and rapid to evaluate acrosomal morphology in bull and boar spermatozoa under field condition, however, in buffalo bull, only have been used to evaluate the sperm vitality. SpermBlue® (SB) is a new stain amenable to automated sperm morphology analysis in several species, but not has been proved in buffalo. This trial was aimed at exploring a simple procedure to compare two methods to evaluate buffalo bull sperm for identification of acrosomal damage after cryopreservation. Three straws of frozen semen from five Murrah buffalo bulls were thawed, smeared, and stained with EN and SB, then evaluated under a light microscope with immersion objective (x100). Student's t-test and Pearson correlation were used to compare the values of acrosome damage between stains. The results showed high percentages of spermatozoa with intact acrosome after cryopreservation, however, a significant difference ($P < 0.0001$) was observed when the two stains were compared. The percentages of spermatozoa with acrosomal loss detected by SB (13.05 ± 1.11) were consistently higher than those obtained by EN (2.25 ± 0.4) and a poor correlation ($r = 0.43$, $P > 0.05$) between both stains was found. The highest percentage of spermatozoa with apical ridge and bright line at the apex of the head representing the cells with intact acrosome were observed with EN stain; while with SB intact acrosome were dark blue. In conclusion, SB stain is better than EN to evaluate the acrosome integrity in frozen-thawed buffalo sperm, because offers more reliable results. Probably the components of extender used in this trial affected the response of EN stain.

Keywords: cryopreservation, spermatozoa, viability.

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Seasonality and influence on birth of females buffaloes

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The buffalo is one of the species most rusticity in livestock production systems, integrates harmoniously with the ecosystem through their products (milk and meat), animal traction and transport among others, but one of its major drawbacks is the stationary of births, which production was not consistent especially difficult to market milk and meat, for lack of continuity in the supply markets. The aim of this study was to evaluate the seasonality of first births of females buffaloes occurred in the last 18 years in different areas of Colombia. A total of 4,112 females with first birth records, distributed at different areas of the country such as, Puerto Berrio, Yondo, Puerto Nare, the Opon, Caquetá, Arjona Bolívar, Sitio Nuevo Magdalena, Cesar Pelaya, Mojana sucreña and Pivijay Magdalena. They were evaluated during the period 1999- 2012, they were grouped into three groups aged 27 and 34 months and 39 months and 36 43 and 61 months, respectively. The distribution of births in different months of the year shows a higher concentration of them in the months of August, September, October and November; presenting the highest value in August with 22% a reduction for the months of February, March, April and May was presented, presenting as a lower value 3% in February. No significant difference for the groups according to the age of the animals was found. Additionally seasonality for heifers (first birth) and buffalo more than one birth using 2,067 females records third birth was compared, obtaining that for heifers the month increased occurrence of birth was August and for buffalo cows third delivery was November with regard to less occurrences was February and May, respectively, for each batch of buffaloes first and third lactation. This study shows that there is seasonality in the first and third birth concentrated between the months of August and November which corresponds to a time of transition from summer to winter in areas evaluated.

Keywords: reproductive efficiency, Environmental, Seasonal changes.

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Prospective for the innovative welfare index of the Mediterranean Italian Buffalo population

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The aim of this study was to evaluate preliminary morphological traits to be considered as factors for the evaluation of buffalo welfare at farm level. Data of heel height (HH), Body condition score (BCS), udder function (UF), Legs and Feet (LF) and Milk Yield (MY) were collected from 763 Mediterranean Italian Buffalo belonging to five herds in the Campania region by two linear type trait evaluators in the year 2016. Presence or absence of overgrowing claws was also reported in the file. Evaluators assigned linear scores to Udder Function (UF) and Legs and Feet (LF) traits using a continuous scale from 1 to 5 points. BCS was evaluated using a continuous scale from 0 to 9 points; whereas Heel Height (HH) was measured by the evaluators directly from the animal. Presence or absence of overgrowing claws was also reported for each evaluated animal. Frequency, number of records and unadjusted average for the analysed traits were obtained using the PROC ANOVA procedure of SAS (SAS, 2005). Assessment of the target BCS during lactation can be helpful in determining whether the nutritional program and other management practices related to the animal welfare are adequate. BCS can be used as measure of buffalo welfare. Preliminary results showed an effect of HH on claws overgrowth, as an important factor to consider in the setting up of the Buffalo Welfare Index. Also UF is of great importance from the point of view of milk production, its quality and overall buffalo welfare. Healthy udder and teats decrease the incidence of intramammary infections. These factors impact dairy buffalo involuntary culling and contribute to prolong their productive life. Next step in the study will be to weight these factors affecting buffalo welfare for their economic value.

Keywords: animal welfare, Buffalo Welfare Index, buffaloes.

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Effect of different extenders and low density lipoprotein concentrations on sperm motility of frozen-thawed sperm of water buffaloes

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This work aims to compare the effects of different extenders containing either one of two low density lipoprotein (LDL) concentrations in replacement of whole egg yolk on the post-thaw motility of buffalo sperm. One ejaculate of six Murrah buffalo bulls kept under a weekly artificial vagina seminal collection regimen was used. Ejaculates with total motility higher than 70% and $\leq 30\%$ abnormal sperms were diluted to 50×10^6 spermatozoa/ml with six different extenders: Tris-fructose-citric acid containing LDL 5% or 10%; Tris-glucose-citric acid containing LDL 5% or 10% and Tes-tris-fructose containing LDL 5% or 10%. Diluted semen aliquots were cooled ($-0.25^\circ\text{C}/\text{min}$) and equilibrated at 5°C (5 hours in total) and then submitted to the freezing-thawing process. Sperm total (TM) and progressive motility (PM) were evaluated after thawing at 0, 30, 60, 90 and 120 minutes of incubation at 37°C , using the Sperm Class Analyzer. Statistical analyses were carried out at a significance level of 5%. TM and PM gradually decreased over time after thawing. TM was similar between extenders at any evaluation time. Values of TM ranged from $56.8 \pm 18.2/73.9 \pm 14.1$ (min/max) at time zero to $38.2 \pm 8.2/51.4 \pm 11.8$ (min/max) at 120 minutes post-thaw. At time 0 after thawing, PM was similar between extenders, but after 30 minutes of incubation, including time 120, the Tes-tris-fructose-LDL 5% extender was inferior in maintaining PM ($p < 0.05$). Values of PM ranged from $31.8 \pm 15.9/41.15 \pm 11.0$ (min/max) at time zero to $12.9 \pm 9.7/28.5 \pm 16.1$ (min/max) at 120 minutes post-thaw. In conclusion, the extenders based on Tris-citric acid either with fructose or glucose and Tes-tris-fructose-LDL 10% were equally effective in preserving post-thaw motility of water buffalo spermatozoa.

Acknowledgment: This study was supported by the Instituto Nacional de Ciência e Tecnologia (INCT-Pecuária), CNPq, FAPEMIG and CAPES.

Keywords: *Bubalus bubalis*, cryopreservation, CASA.

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Canadian experience with artificial insemination in water buffalo

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Canadian Buffalo production systems is beginning, there are big problems to get animals for improvement making artificial insemination (AI) an alternative, additional AI is the best tool for genetic improvement. The aim of this paper is to show the Canadian experience in a buffalo farm located in Stirling, Ontario Canada, during 2014-16. Age, parity, days open, protocol and bull used has been recorded. Semen from 20 different Mediterranean bull was used. In the cases that apply comparison of proportions between groups were performed using (Software Epiinfo V 6.0). Data from It has been recorded 224 inseminations, 194 (85%) synchronized and 26 in natural cycle 26 (15%). Average of age, parity, open days were 5.15 years, 2.9 calving, 170 days respectively. Pregnancy rate was 51% and 61% with no statistical difference between the two treatments. 9% of the pregnancies ended before 150 days all of them from the synchronized group. 16% of the animals were recorded as non responders to the synchronization protocol. No statistical differences in the pregnancy rates between bulls were observed. This is the first report of AI in Canada, showing a young group of animals with good reproductive performance. Pregnancy rates are higher than those obtained in Colombia. Abortion must be taken in account because the possibility of embryo mortality is part of the programs located far from the tropics as European researchers has been reported. Similar findings regarding non response to treatment is in agree from other studies of our group. The lack of statistical significance between treatments could be attributed to the management of the farm. The results show the feasibility of using AI to introduce genes to a buffalo population in this country, allow to propose selection strategies to increase buffalo production in this country.

Keywords: buffalo, insemination, synchronization, estrus.

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Perfiles de progesterona en búfalas de agua sometidas a diferentes protocolos de sincronización de ovulación

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En Colombia se ha utilizado principalmente la Inseminación a tiempo fijo, con diferentes esquemas de sincronización de ovulación, obteniéndose bajos porcentajes de preñez. El protocolo más conocido es Ovsynch, que se ha propuesto para ser usado en la estación reproductiva favorable de los búfalos. El objetivo del presente trabajo fue evaluar el comportamiento de la progesterona durante el proceso de sincronización de celo durante la estación reproductiva 2015-2016. Se utilizaron 37 búfalas paridas en ordeño, las cuales fueron asignadas al azar en dos tratamientos. T1: sincronización con implante de progesterona; T2: protocolo Ovsynch. Se tomaron muestras de sangre para determinar P4 por la técnica de ELISA, el día del inicio del tratamiento (0), de la inyección de prostaglandina (7), Inseminación (10), y 5 días postovulación (15). Se utilizó un diseño completamente al azar con un arreglo de medidas repetidas en el tiempo, con una estructura de varianza de simetría compuesta. La tasa de preñez se evaluó con una prueba Ji-Cuadrado. Se consideró diferencia significativa $p < 0,05$. No se encontraron diferencias significativas en los niveles de progesterona ($p=0,07$) y en las tasas de preñez ($p=0,693$) entre los tratamientos. Los niveles de progesterona fueron 3.111, 2.827, 0.476 y 2.1145 ng/ml para los días 0, 7, 10 y 15, respectivamente. Se encontraron diferencias significativas entre los días 0 y 7 con el día 10 ($P=0,0001$) y entre los días 10 y 15 ($p=0,0106$). Se pudo demostrar que durante la estación reproductiva el protocolo de sincronización no tiene un efecto sobre los niveles de progesterona; esto explicaría en parte que no haya diferencias en las tasas de preñez. Se encontró una disminución en los niveles de P4 después de la inyección de prostaglandina, mostrando la adecuada respuesta al medicamento. En el día 15 se observó que el cuerpo lúteo no alcanza aún su madurez.

Palabras clave: progesterona, estación, protocolos, dinámica hormonal.

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Genome-wide association studies for milk production traits in Mediterranean water buffalo

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Water buffalo is the second largest resource of milk supply around the world. Buffalo milk is well known for its special quality in terms of fat, protein, lactose, vitamins and minerals content. However, the total yield of buffalo milk is very low, and limited studies focused on the improvement of milk production, especially using advanced genomic selection technology. The present study aims to investigate the genomic markers that are associated with buffalo milk production traits, and the potential of genomic selection for improving milk production. Six milk production traits were collected from 489 Mediterranean water buffalo with a total of 1408 lactations. Genetic parameters were estimated using an animal model implemented in ASReml. Genotyping was conducted using the 90K Affymetrix Axiom[®] Buffalo SNP Array. Following quality control, a total of 60,387 SNPs and 462 animals remained for the genome-wide association studies and the multiple test of P-value was adjusted by genome wide False Discovery Rate (FDR) correction. The heritability estimates for all of the studied traits ranged from 0.19 ± 0.09 to 0.38 ± 0.11 . Two regions found to have an effect on buffalo 270-days fat yield and protein percentage are located on bovine chromosomes (BTA) 3 and 14 which are homologous to buffalo chromosomes (BBU) 6 and 15, respectively. We identified 29 SNPs associated with the milk production traits at $P \leq 10^{-4}$ and 4 significant SNPs at $FDR \leq 0.10$. These findings provide useful information about genomic regions affecting buffalo milk production traits, and show the potential for improving breeding programs using a genomic approach. However, a more precise buffalo genomic map is required to identify the genes affecting the traits, and a larger sample size may help to improve the detection power.

Keywords: buffalo, milk production, genetic parameters, GWAS.

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Seasonal variations in expression intensities of seminal plasma proteins of buffalo semen : preliminary results

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Introduction

The aim of this study was to evaluate the fluctuation of protein content in seminal plasma in buffaloes kept in a tropical climate, during the dry and rainy season. The animals were kept at the Biotechnology Center of Buffaloes, Brazil (19°S and 44°W). The experimental periods was characterized by 5,1mm and 501,0 mm total rainfall, 48,8% and 65,8% average moisture and 21,5°C and 23,9°C average temperature, respectively for the peak of dry and rainy period selected. Twelve ejaculates (two per Murrah buffalo bull) collected in dry and twelve collected in rainy period were selected for the study. The ejaculates were collected with artificial vagina. After semen collection, analyses of subjective motility, vigor, force tourbillon, sperm concentration (Neubauer chamber) and sperm morphology (phase contrast microscopy) were performed. The seminal plasma was harvested from semen samples by centrifugations at 4°C (700xg for 15 min. followed by 10.000 xg for 1 h) and total protein was estimated by Bradford's method. Seminal plasma proteins were subjected to SDS-PAGE. Gels were then stained with Coomassie blue and analyzed using QuantityOne software (BioRad,USA). All variables were compared by ANOVA followed by Tukey test (P <0.05). There were no differences on seminal parameters of fresh samples, whether collected during the drought season with no heat stress or during the rainfall season associated with a moderate heat stress. Analysis of the gels revealed that six protein bands showed higher expression intensities in the rainy period: band 28.6kDa, 25.5kDa, 24.2kDa, 20.8kDa, 18.6kDa e 16.7kDa. It was evident that expression of seminal plasma proteins in buffalo bulls, particularly low molecular weight proteins, is influenced by climate changes. Further studies are required to validate the exact role of each protein plays in sperm quality.

Acknowledgment: This study was supported by the Instituto Nacional de Ciência e Tecnologia (INCT-Pecuária), CNPq, FAPEMIG and CAPES.

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La inseminación Artificial Tiempo Fijo, el camino para el mejoramiento del Búfalo Mediterráneo en Venezuela

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Vista la importancia que tiene el crecimiento bufalino en nuestro país, establecimos un camino donde involucramos a un equipo de colaboradores para el proyecto de llevar a nuestros búfalos mestizos a sus razas originarias. Usando como metodología, la Inseminación Artificial Tiempo Fijo (I.A.T.F.), en un periodo de un año a 3000 búfalas en 7 provincias del país y con un total de 10 criaderos, los protocolos Ovsynch+resynch, Dispositivo Intra Vaginal (DIV)+Resynch, en Búfalas Posparto y Buvillas, también involucramos la monta natural controlada por 60 días para medir los porcentuales de gravidez al posparto. En los grupos involucrado de productores, como norma 45 días previo al inicio de los trabajos, unificamos los criterios en los planes sanitario para el control de enfermedades reproductivas, mineralización, instalaciones para el manejo de los grupos, evaluación de pasturas y nutrición, selección por definición de caracteres raciales a la raza Mediterránea y por ecografía la evaluación ginecológica. Los grupos de búfalas fueron de 20 animales que de acuerdo a la temporada de mayor fertilidad se les aplicarían los protocolos de Ovsynch+Resynch y (DIV)+Resynch, para temporadas de baja fertilidad. A su vez utilizamos grupos testigos donde en el pos-parto solo estuvieron en monta natural por 120 días para medir las tazas % de gravidez de los Butoro. Los resultados alcanzados en el periodo estimado fueron finalmente 1864 búfalas inseminadas para un total de 60% de gravidez con IA, mas el complemento de los 60 días de monta natural controlada, los resultados en estos grupos fue de 82 % de gravidez. Comparando con dos grupos testigos donde intervinieron cuatro Butoro en monta natural durante 4 meses, en ellos nos reflejaron cifras de gravidez similares al 78%.

Keywords: inseminación artificial, I.A.T.F, dispositivo intravaginal, búfalos.

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Impacto en la productividad de sistemas bufalinos mediante el diagnóstico temprano de gestación con pruebas sanguíneas de incubación corta y lectura visual

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La determinación temprana de la gestación es un reto dentro del manejo del hato, ya que disminuir los días abiertos tiene un alto impacto en la eficiencia y rentabilidad del negocio ganadero. En hembras rumiantes mediante la aplicación de una prueba de ELISA cualitativo para las proteínas asociadas a la gestación se puede determinar gestación a partir del día 28. El objetivo del presente trabajo es validar la prueba diagnóstica denominada IDEXX Rapid Visual Pregnancy Test en la especie *Bubalus bubalis*. Este trabajo se llevó a cabo entre febrero y agosto del 2016, en el laboratorio de Biotecnología Animal de la Universidad Nacional de Colombia. Se tomaron 234 muestras de sangre completa y 234 muestras de suero de bufaleras de 5 zonas de Colombia, en el mismo momento de la toma de la muestra se realizó ultrasonido para diagnóstico de gestación. La sangre extraída fue procesada mediante el uso del IDEXX Rapid Visual Pregnancy Test® (Cat No 06-41369-00), de acuerdo con las instrucciones del fabricante. Con base en las muestras analizadas, se calculó la sensibilidad y especificidad de la prueba. La sensibilidad en plasma y suero fue de 83,7% y 94,17% respectivamente, mostrando una mayor capacidad de la prueba para detectar preñez temprana. La especificidad de la prueba con plasma y suero fue 89,9% y 85,5%, respectivamente, lo que indica la capacidad de la prueba para detectar búfalas vacías. La exactitud de la prueba en plasma fue del 86,32% y para el suero fue del 89,32%. Se concluye que el uso del kit IDEXX Rapid Visual Pregnancy Test sirve como herramienta complementaria para identificar el estado gestacional de las hembras y es base para la toma de decisiones, su rapidez y versatilidad la hace prueba fácil de usar, aún en las condiciones de campo.

Palabras clave: días abiertos, intervalo entre partos, proteínas asociadas a la gestación, herramienta.

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Persistencia de los niveles de proteínas asociadas a la gestación en el posparto de las búfalas de agua (*Bubalus bubalis*)

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Las proteínas asociadas a la gestación (PAG) pertenecen a una familia de proteinasas aspárticas inactivas, que son producidas principalmente por las células binucleadas de la placenta. Están formadas por un grupo de 22 genes localizados en el cromosoma 29 de los vacunos), están tienen diferentes patrones de expresión durante la gestación. Recientemente se han desarrollado pruebas para el diagnóstico de preñez mediante ensayos inmuno-enzimáticos (ELISA) que detectan proteínas asociadas a la gestación (PAG), que son seguras para detectar preñez a partir del día 28 pos- servicio, sin embargo no existe información sobre la dinámica de las PAG en el posparto temprano de búfalas de agua. El objetivo del presente trabajo fue evaluar el momento postparto en el que los niveles de PAG desaparecían de la circulación. Este trabajo se llevó a cabo durante los meses de Abril y Junio de 2016, en una bufalera localizada en Chigorodó, Antioquia, Colombia. Fueron escogidas 6 búfalas recién paridas durante la misma semana, a cada una se le tomo una muestra de sangre con y sin anticoagulante semanalmente hasta la semana 7 postparto, para la determinación de los niveles de PAG se utilizó el IDEXX Rapid Visual Pregnancy Test® (Cat No 06-41369-00), de acuerdo con las instrucciones del fabricante. Se pudo observar que hasta las 11 días el 100% de los animales presentaron niveles detectables de PAG, para el día 21 el 50% y para el día 35 el 100% de los animales fueron negativos y se mantuvieron negativos hasta el día 46. Se muestra por primera vez en la literatura la dinámica de los niveles de PAG en búfalas, la desaparición de los niveles después del día 35 permiten su uso seguro en programas de reproducción en los post parto temprano de los animales.

Palabras clave: PAG, posparto, dinámica, ELISA.

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Estimation of linkage disequilibrium pattern of a Bulgarian Murrah buffalo population in the Philippines

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The use of conventional best linear unbiased prediction of estimated breeding values (BLUP EBVs) as a tool for ranking animals for culling/selection in the dairy buffalo breeding program of the Philippines has resulted significant gains in increasing milk production potential of the local dairy buffalo population. The availability of dense panel of single nucleotide polymorphism (SNP) markers for the water buffalo species provides an opportunity to further increase the rate of genetic gain through marker assisted selection (MAS) either by identifying markers significantly associated with milk production traits using genome wide association study (GWAS) or genomic selection (GS). However, both GWAS and GS depend on markers to be in linkage disequilibrium (LD) with quantitative trait loci (QTL). As the local dairy buffalo population has not been subjected to either methods, there is a need to estimate the extent of LD in the population. For LD estimation, 59 unrelated, neither half-sib nor full-sib, Bulgarian buffalo animals were genotyped using the Axiom Buffalo Genotyping Array. Generated "CEL" files were analyzed using the Axiom Analysis Suite software. For downstream analysis, the extent of LD expressed as r^2 , was estimated using the R package "synbreed" with a gateway to the PLINK software. To visualize LD decay, the r^2 values were plotted against pairwise distance of increasing interval. The proportion of marker pairs in each chromosome with $r^2 > 0.20$ ranged from 0.58% to 1.36%. The average marker interval 43.6 kb with the largest and smallest gap located in chromosome 12 and 15, respectively. The average r^2 for SNP pairs at ~44kb was 0.24. The longest interval with useful LD ($r^2 \geq 0.20$) is at 60-70kb. There is sufficient LD in the local dairy buffalo population suitable for GWAS and GS studies

Keywords: dairy buffaloes, Genomic selection, Linkage disequilibrium, SNP panel.

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Follicular and luteal dynamics of buffalo heifers

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The aim of this study was to ascertain the number of follicular growth waves, the growth dynamic and the vascularization of the corpus luteum (CL) during the estrous cycle of Murrah buffalo heifers. Buffalo heifers (n=12) 28.8±1.5 months old, 387.2±18.5 Kg and with BCS 4.0±0.1 (scale 1–5) were submitted to two PGF2 α injections (150 μ g im D-Cloprostenol, 11 days apart, Sincrocio[®], Ourofino Agronegócio). Ultrasound examinations were performed after the second PGF2 α injection (24/24h for 50 days, DP2200Vet, Mindray) to establish the day of the first ovulation (Day 0; D0), to quantify the follicular growth waves, to measure the follicles and the CLs and, to verify the day of the second ovulation. The CLs vascularization was assessed from D2 by color Doppler ultrasonography (M5Vet, Mindray). Only data from cyclic heifers (n=5; two ovulations after the second PGF2 α injection) were considered on the analysis. Data are expressed as mean±SEM. The duration of the estrous cycle was 23.6±1.4 days, with 3.2±0.2 follicular growth waves (4 heifers showed 3 waves and 1 heifer one wave). The follicular emergence day, the number of follicles <8mm on the emergence and the maximum diameter of the follicle on the first, second, third and fourth waves were, respectively: 0.0±0.0, 38.6±1.5, 10.6±0.5mm; 6.8±1.1, 34.4±5.3, 9.6±0.8 mm; 12.4±1.5, 33.4±4.8, 13.3±1.0 mm; 15.0, 29.0, 13.8mm. The CL diameter increased from D1 to D8, which remained stable until D17, with posterior regression. Similarly, the CL vascularization increased from D2 to D6, which stabilized until D17, with posterior regression. It was concluded that Murrah buffalo heifers have on average 23 days of estrous cycle, 3.2 follicular growth waves and active morphological and functional CL until D17 of the estrous cycle.

Keywords: follicle, corpus luteum, emergence.

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Effect of follicular growth superstimulation anticipation on embryonic structures recovery of superovulated buffaloes

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The aim of this study was to evaluate the effect of follicular growth superstimulation (FGS) anticipation on follicular and superovulatory responses, and on embryonic structures recovery rate of superovulated buffaloes. Buffaloes (n=6; in a cross-over experimental design) received an intravaginal progesterone device (P4, 1.0 g, Sincrogest®) plus estradiol benzoate (EB, 2.0 mg im, Benzoato HC®) at random stage of the estrous cycle (Day 0; D0). On D3, buffaloes were randomly allocated to one of two groups and treated as follows: GD3 (n=6), FGS initiated on D3 (333 IU im FSHp/LH, Pluset®) twicedaily, in 10 decreasing doses; and GD4 (n=6) FGS initiated on D4 (333 IU im FSHp/LH, Pluset®) twice-daily, in 10 decreasing doses. On D7, the intravaginal P4 device was removed and buffaloes were given PGF2 (150 µg im D-Cloprostenol, Croniben®) on D6 and D7. On D8, the animals from two groups received GnRH (20 µg im buserelin acetate, Sincroforte®). The artificial inseminations were performed 12 and 24 h after the induction of multiple ovulations. Ultrasound examinations were performed on D0 to evaluate the cyclicity, D3 to verify the ovarian follicular population, D8 to ascertain the follicular response to the superstimulation, and on D15 for accounting the multiple ovulations. The embryonic structures were collected on D15. Binomial and continuous variables were analyzed by GLIMMIX and MIXED procedures of SAS®, respectively. There were no significant differences between GD3 and GD4 for the number of follicles ≥ 3 mm on D3 (29.8 ± 3.8 and 22.0 ± 1.7), number of follicles ≥ 8 mm on D8 (7.5 ± 2.3 and 7.5 ± 1.5), number of CL on D15 (4.0 ± 1.9 and 3.3 ± 1.0), ovulation rate (51.8 and 52.7 %) and embryonic structures recovery rate (13.6 and 18.1 %). It was concluded that the FGS anticipation do not increase the follicular and superovulatory responses, and the embryonic structures recovery rate of superovulated buffaloes.

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Keywords: embryos, follicular emergence, superovulation.

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Heritability and correlations of welfare-related type traits and milk yield in the Mediterranean Italian Buffalo population

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The objective of this study was to estimate heritability and correlations among welfare-related type traits of Udder Function (UF), Feet and Legs (FL), BCS, Heel Height (HH) and Milk Yield (MY) for Mediterranean Italian buffalo, to assessing their impact on buffalo Welfare. A total of 5,575 records for BCS, UF, FL, HH, and MY, scored from 2004 to 2015, were extracted from the Italian Buffalo Breeders Association database. Type traits were scored using a scale 1-50 (HH), 50-100 (FL, UF), and 1-9 (BCS). Buffalo in the analysis had both parents identified by DNA-testing. The BLUP-Animal Model was used for the analysis, with the fixed factors of Herd-Year, Evaluator-Year, Age-Parity, Days from calving to evaluation, and with random factors of animal and residual. Two-trait analyses were performed to estimate correlations among traits. The most heritable traits were BCS (0.27 ± 0.06) and HH (0.23 ± 0.04), whereas the least heritable traits were UF (0.12 ± 0.03) and FL (0.13 ± 0.03). MY showed the largest heritability (0.45 ± 0.04). The low type traits heritability, may reflect low genetic variability in the population. However, producers should select for those traits to improving welfare. Among traits, genetic and environmental correlations tend to be zero, except for genetic correlation of UP vs FL (0.74 ± 0.08) and UP vs BCS (0.60 ± 0.02). MY and HH showed a positive correlation of 0.12 (-0.01 ± 0.04). Slightly negative genetic correlations were found for FL vs HH (-0.04 ± 0.04), FL vs MY (-0.01 ± 0.04), and BCS vs MY (-0.01 ± 0.02). This results showed that type traits can be used as weighted factors for a future welfare buffalo index. Selecting for optimal type traits, will reduced the loss of buffalo due to involuntary culling. Further investigations on correlations of type traits and longevity are needed to better understand their use as indicator of buffalo welfare.

Keywords: Mediterranean Italian Buffalo, animal welfare, heritability, Welfare Buffalo Index.

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Effects of season on pregnancy rates and milk progesterone profiles in Water Buffalo in Canada

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A growing market in Canada is the use of Water buffalo products, such as the high quality milk they produce. Although they are capable of breeding throughout the year in temperate climates, they show seasonality in reproductive efficiency. Being recently introduced to Canada, the effect of the environment on seasonality of water buffalo reproduction is not yet understood. The present study aims to determine the effects of season on pregnancy rates and estrous cycle in water buffalo in Canada at a latitude and longitude of 44.4 N and 77.6W. Fertility data from timed insemination and natural breeding was collected over two years and pregnancy rates were calculated for Fall/Winter and Spring/Summer. Estrous cycle length and regularity was measured by milk progesterone analysis. Milk was collected from randomly selected buffaloes during winter (n=5) (November 25/2014 - January 2/2015) and summer (n=5) (May 21/2014 - June 21/2014). Progesterone was measured every other day via ELISA. Pregnancy rates in Spring/Summer (28.9%) were significantly lower than pregnancy rates in Fall/Winter (53.4%). Summer months showed abrupt rise and fall in progesterone with 2-day elevated intervals. Winter months showed increased progesterone with peaks persisting for 10 days, during the 20-22 day estrous cycle. Results show, in winter and fall (traditional breeding season) the estrous cycles were regular with anticipated pattern and pregnancy rate was significantly higher compared to summer and spring (traditional low-breeding season) with irregular estrous cycles. Average progesterone levels during the breeding seasons were higher indicating presence of an active corpus luteum.

Keywords: water buffalo, quality milk, buffaloes.

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Fixed Time Artificial Insemination (FTAI) in buffalo using different estrus synchronization protocols

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The objective was to demonstrate the efficacy of ear implant and intravaginal progesterone device to synchronize estrus in female buffaloes (n=180) and use fixed time artificial insemination (FTAI) associated or not to GnRH. The buffaloes were raised and grazed in rotational artificial pastures supplemented with a commercial mineral mixture and water supplied *ad libitum*. Four groups with 45 females were established: group (G1) and G2 received ear implant, and beside that group G2 received 1 ml of GnRH (i.m.) at the time of insemination; group 3 and 4 received vaginal implant and group 4 was received 1 ml of GnRH i.m. at the time of insemination. Females were inseminated 60 hours after with drawal of progesterone implant. The protocol efficacy, for GnRH use, type of implant and the AI technician cleverness on pregnancy rates were evaluated and compared. Data collected were analyzed by chi-square statistical test, with 5% significance. The overall pregnancy rate was 46.6% (84/180), and 40.0%, 60.0%, 53.3% and 33.3% for G1, G2, G3 and G4 respectively, with statistic difference for pregnancy rate between G2 and G4 ($P < 0.05$). Meanwhile isolated evaluation of GnRH influence at the time of insemination did not show any statistical difference ($P > 0.05$) between G2 and G4 pregnancy rate of 46.67% (42/90). Regarding the type of implant on pregnancy rate it was 50% (45/90) and 43.33% (39/90) respectively, also without any statistical difference ($P > 0.05$), however the AI technician cleverness influenced the pregnancy rate ($P < 0.05$), being 37.93% (33/87) and 54.84% (51/93) for AI technician A and B, respectively. Therefore, the GnRH application associated with the implantation of auricle progesterone device produced better results.

Keywords: buffaloes, GnRH, progesterone.

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Identification of sperm subpopulations in water buffalo ejaculates: changes in cryopreservation stages and bull variation

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The objective of this study is to identify and characterize the sperm subpopulations existing in water buffalo semen using a computer assisted sperm analyzer (CASA), assess the effects of cryopreservation on the sperm subpopulation structure and evaluate bull variability. Eight Bulgarian Murrah bulls were collected with semen, four times in an interval of one week each. The semen was cryopreserved following a standard protocol and sperm kinematics was assessed. Clustering methods were applied to individual sperms forming two significantly different ($P < 0.05$) subpopulations. Subpopulation 1 represents those spermatozoa that moved most rapidly and progressively (46.29%) and Subpopulation 2 includes spermatozoa with relatively low velocity or poorly motile but with high progressiveness (53.41%). There is a decline on the population of Subpopulation 1 sperms from fresh (52.52%) to pre freeze (45.73%) to post thaw (35.17%) stages and significant difference on the sperm kinematics between subpopulations. A significant decline in the values of distance, velocity and ALH parameters were observed at post thaw, while an increase is observed on trajectory and BCF kinematics. Values of sperm kinematics are also significantly different ($P < 0.05$) among all bulls. The frequency distribution of spermatozoa on both subpopulations was quite similar for all bulls in pre-freeze and post-thaw stages but with significant ($P < 0.05$) variability on fresh stage. Bulls with the highest maintained frequency of Subpopulation 1 sperms are denoted as good freezer bulls. In sum, kinematic characterization of water buffalo sperm and clustering into subpopulation enabled identifying bulls that are more resistant to cryopreservation and production of quality semen for genetic propagation.

Keywords: sperm subpopulations, buffalo semen, sperm kinematics, cryopreservation, CASA.

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Performance of the Axiom 90k Buffalo Genotyping Array in four Philippine water buffalo populations

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The Axiom Buffalo Genotyping Array, a high-density single nucleotide polymorphism (SNP) chip for water buffalo (*Bubalus bubalis*) was used on four water buffalo breeds in the Philippines to test the suitability of the SNP chip on the local buffalo population. A total of nine hundred eighteen (918) DNA samples from both male and female buffaloes were submitted for genotyping. The Axiom analysis suite software was used to analyze the raw data for quality control metrics and generating genotype calls. For downstream analysis, principal component analysis (PCA) and heatmap based on genomic relationship matrix (GRM) using R was done to visualize the relationship structure among the four buffalo breeds. Polymorphic (PHR) SNPs useful for downstream study were identified for the four Philippine water buffalo populations using the Axiom 90K Buffalo Genotyping Array. The number of polymorphic markers for the 3 riverine breeds is higher ranging from 57,094 to 67,810, compared to those of the Philippine swamp population with only 16,573 PHRs since the SNPs included in the array all came from riverine breeds. The genotyping array will be useful for genomic studies in the four buffalo populations. However, for the swamp population with a longer inter-marker distance in autosomes, the array's usefulness will be limited to population diversity studies. Common PHR SNPs among the three riverine breeds was determined to be 46,445. There were 10,443 PHR SNPs common to the four populations and could serve as a basis for the design of a lower density SNP chip specific for Philippine buffalo populations. The PCA plot and heatmap generated from GRM using only the PHR SNPs clearly separated the riverine and swamp populations and showed the genetic relationship among the riverine breeds.

Keywords: water buffaloes, Axiom Buffalo Genotyping Array, PCA plot.

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Fourier harmonic analysis in comparison with hypoosmotic swelling test and computer assisted sperm analysis in examining the quality of water buffalo bull semen

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The water buffalo industry needs quality control standards to identify high fertility bull semen prior to distribution for artificial insemination programs. Fourier harmonic analysis or FHA is a mathematical tool used in analyzing the sperm DNA defects by analyzing the sperm nuclear shape and is proven effective in predicting fertility in bulls and boars. Hypoosmotic swelling test or HOST is a test that assesses the functional integrity of sperm plasma membrane. Computer assisted sperm analysis or CASA is a tool that assesses the motility characteristics of sperm cells. Comparative studies were done to assess the similarity and differences of these tests. Semen samples from 44 buffalo bulls were individually processed and subjected for FHA, HOST, and CASA. Of the 44 bulls, FHA results classified 12 high fertile, 22 mid fertile and 10 low fertile bulls, respectively. Data on HOST and CASA were analyzed for each bull-class to assess differences. Results of HOST showed that functional integrity of bulls classified as High Fertile (80.4 ± 2.1) was comparatively closed with Mid Fertile (81.2 ± 2.0) but these are significantly higher ($P < 0.05$) than the Low Fertile Group (71.8 ± 3.7). However, results in CASA showed no difference on the General and Progressive motilities of High (62.6 ± 2.0 and 51.4 ± 2.5), Mid (64.9 ± 1.5 and 54.4 ± 1.8), and Low (63.7 ± 1.9 and 53.5 ± 2.2) Fertile bulls. These results suggest that the FHA and HOST have the same potential in assessing water buffalo bull sperm quality. Confirmation by AI and IVF is underway to determine the efficiency of these tests in assessing bull fertility.

Keywords: membrane integrity, semen quality, sperm DNA, sperm nuclear shape.

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El conocimiento como base del mejoramiento de la producción bufalina en el país: estudio de caso programa de inseminación artificial 2015-2016

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Establecer sistemas productivos, bufalino para, la producción de carne, leche y trabajo, se basan en la obtención de información y derivado de su evaluación se pueden establecer objetivos claros para proyectar la producción de la especie en el país. Con el apoyo de la Asociación Colombiana de Criadores de Búfalos la Universidad de Antioquia, el Ministerio de Agricultura y Desarrollo Rural y los criadores, se han realizado pruebas de comportamiento, se publicó el catálogo con los valores genéticos de reproductores que puede ser utilizado para mejoramiento. El presente trabajo prospectivo tiene como objetivo describir los hallazgos sobre información de hatos bufalinos localizados en diferentes regiones del país. La información colectada por los técnicos de la asociación que participaron en el programa de asistencia técnica fué realizado en 5 departamentos del país durante la temporada reproductiva 2015-2016, Se hizo énfasis en parámetros como identificación de los animales, raza, estado fisiológico, condición corporal, edad, producción de leche, días abiertos. Se analizaron registros de 1162 búfalas, en 34 hatos, el 50% de los animales tenían registros productivos. Las búfalas presentaron en 70,9 meses de edad, 91% de los animales fueron caracterizados como mestizos, con 2,82 partos y entraron al programa de inseminación con 83 días abiertos, condición corporal 3,7 (escala de 1 a 5), y una producción láctea promedio de 4,53 kgr con un rango entre 1 y 10 L de leche. Los parámetros de producción se confirman con la información oficial existente derivada del control lechero. Se observa un hato joven, no existe un componente racial definido y con unos parámetros establecidos, lo permite plantear con bases sólidas el programa de mejoramiento genético y proyección de la producción bufalina en nuestro país. Es necesario incrementar el número de hatos que lleven registros productivos para tener mayor impacto en la producción.

Palabras clave: inseminación, programas, desarrollo rural, mejoramiento.

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Sanitation

Characterization of the gastrointestinal microbiota of mediterranean water buffaloes

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Gut microbiota plays a key role on host wellness by modulating the immune response and influencing the onset of diseases and disorders. Recently, some studies have investigated the complexity of microbial communities resident in the intestine of steers and pre-weaned calves. There are no data on the characterization and on the influence of diet on the gastrointestinal microbiota of the Mediterranean water buffalo. This study characterized the microbiota of water buffalo rumen, gut and feces by 16S rRNA analysis using a Next Generation sequencing approach. To evaluate the impact of the diet on gut microbiota, the study also included a group of animals fed with tomato peels added to the traditional feed. Our results highlight the heterogeneity of microbial communities resident in the different areas of the gastrointestinal tract, and provide insight on the effect of the diet on gut microbiota.

Keywords: diet, metagenomics, tomato peels.

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Foremilk samples: bacteriological status and somatic cell count in Mediterranean Italian Buffalo cow, proposal of discriminant value between infected and uninfected quarters

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Mastitis is an inflammatory disease in one or more quarters of the udder, that represents one of the most important costly diseases of dairy species. The objectives of this study were to determinate the bacterial pathogens causing sub-clinical mastitis and the relation between somatic cell count (SCC) and bacteriological status of mammary gland in Mediterranean Italian Buffalo cows. Bacteriological status and SCC of 1,273 foremilk samples in Mediterranean Italian buffalo cow, collected from eighteen different herds, located in Latium region (Central Italy) has been evaluated. Receiver-operating characteristic (ROC) curve methodology was applied to evaluate threshold values for detection of better cut-off in SCC to discriminate infected from uninfected udder's quarters. Research funded by PSR Lazio (2007–2013) PIFRL060, Misura 124, MUD 8475907218. Prevalence of infection at quarter level was 20.42%. Between isolated strains, *Staphylococcus aureus* was the most commonly isolated (33.08% of the positive findings), followed by *Streptococcus uberis* (25.77%), Coagulase Negative Staphylococci (20.77%) and *Streptococcus* spp. (13.85%). Average SCC of all samples was 151,000 cell/mL, with significant differences between infected and uninfected quarters (324,000 vs 124,000 cells/mL; $p < 0.001$). The estimated optimal threshold was 311,000 cells/mL, this correct value classified the true positive (42.11%) and true negative (77.49%). This value was similar both for environmental and contagious pathogens (311,000 vs 326,000 cells/mL, respectively). Threshold SCC value could be a valid indirect test to be considered in mastitis prophylaxis program, for microbiological diagnosis at quarter level on Mediterranean Italian buffalo; the lower differences between two cut-off (15,000 cells/mL) suggest that 326,000 cells/mL is a better option for detection of environmental and contagious pathogens. These values should be considered to establish legal limits for buffalo bulk milk in Europe Union as for the cow milk.

Keywords: cut-off, mastitis, ROC curves.

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Seroepidemiology of brucellosis: comparison between buffalos, bovines, goats and sheep from the northeast of Argentina

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Brucellosis is an infectious disease that affects many domestic animals and is caused by a bacterium, *Brucella* spp. Since 1998, SENASA developed a mandatory Brucellosis Control and Eradication National Program that includes bovines and, in 2005, included buffaloes. It is based on S19 *Brucella abortus* vaccination, diagnosis and slaughter of positive animals. The aim of this work was update brucellosis situation in buffaloes, bovines, goats and sheep from Formosa, Argentina. Results of serological test in a reference laboratory (CIT) were analyzed, and a study of prevalence and an epidemiologic survey was carried out in order to establish risk factors for the presence of brucellosis. "Susceptible species" and "flock size" were the two variables selected for the X2 and Odds Ratio tests, in order to determine the risk of occurrence of brucellosis. Positive animals' proportion between 2014 and 2016 were: 1.9 – 6.1%; 0.1 – 0.9%; 1.6 – 0.7% and 0% for buffaloes, bovines, goats and sheep respectively. Prevalence was 14% in buffaloes, 0.2% in bovines, 6.8% in goats and 0% for sheep. The higher prevalence was found in buffaloes, and it was larger in flocks that had 100 to 500 animals than in others ranks. A significant difference ($p < 0.05$) was found among the number of positives buffaloes and other positive species. There was an occurrence of 0.7 positive goats for each positive bovine, while there were 14 positive buffaloes for each positive bovine. The finding of a higher percentage of positives buffaloes to could be caused by the newest incorporation of this specie into control programs and to due to the management difficulties under extensive breeding, characteristic in Argentina. Disease control should be adjusted in buffalo's flocks in order to avoid false-positives or the maintenance of infected animals.

Keywords: *Brucella* spp., epidemiology, serological diagnosis.

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Determination of antibodies against Bovine Leukaemia Virus, *Brucella sp.* and Bovine Viral Diarrhea Virus using the bulk milk Elisa in buffaloes farms in the region south lake Maracaibo, Venezuela

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The bovine leukaemia virus (BLV), *Brucella sp.*, and bovine viral diarrhoea virus (BVDV), among other infectious agents can be found in apparently healthy animals. The use of four commercial ELISA kits in bulk milk samples as a monitoring tool for brucellosis, leucosis and bovine viral diarrhoea, in the region South of Lake Maracaibo was raised. *Materials and methods:* A total of 22 buffaloes farms in the region South of Lake Maracaibo were screened for the presence of antibodies in bulk milk samples against *Brucella sp.*, VLB and BVDV using commercial ELISA kits. *Results:* A determination of antibodies in milk for *Brucella sp.*, 77.27% (n = 17) of herds were negative and 22.73% (n = 5) were positive to I-Elisa. For VLB, a 72.73% (n = 16) of the herds had no antibodies against VLB, and 27.27% (n = 6) were positive. The presence of antibodies to *Brucella sp.*, is dependent on the presence of antibodies against VLB on farms (p <0.01). Only 4.55% (n = 1), they showed total antibodies against BVDV. *Conclusions:* Monitoring in bulk milk, becomes a valuable tool for monitoring infectious diseases, so their use in disease control programs should be included prior validation in the buffalo species.

Keywords: BLV, BVDV, Elisa, buffalo, antibodies.

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Finding and molecular identification of *Fasciola spp.* in buffaloes in the Corrientes Province, Argentina

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In Argentina the NEA region (North East Argentina) holds most of the buffalo heads. Buffalo breeding systems are principally extensive, in natural grass with humid subtropical climate. Current production systems buffaloes placed in different environments of their own, this situation causes loss of rusticity and infestation of parasites of cattle, with which it shares the natural and artificial systems grazing. Preferably evolution in aquatic environments, where the intermediate hosts thrives led the buffalo to infestation by *Fasciola*. Traditionally, the identification of *Fasciola spp.* has been based solely on traditional morphological approaches. However, due to the limitations of these methods, molecular approaches have been developed and used for the identification of flukes and will also prove useful in etiological studies of fasciolosis. The aim of the present work was identification of *Fasciola spp.* affecting buffaloes in Corrientes province. Flukes were recovered from the common bile ducts, confiscated by Flukes, and rinsed thoroughly with warm (37 °C) sterile saline solution to remove bile and/or adhering materials. Samples were preserved in ethanol 99 % at -20 °C until their use. A single-step multiplex PCR was used for testing with the DNA extracted from adult worms, producing amplicons of 1,031 bp for *F. hepatica* and 615 bp for *F. gigantica*. Out of a total of 99 livers, 9 were seized by flukes in the abattoir, 5 of them were processed in the laboratory and 332 flukes were recovered, making an average of 65 parasites per animal. Some random samples were taken, which after being classified as *F. hepatica* by taxonomic characteristics. They were used for molecular studies. The PCR results confirm that flukes are of the *F. hepatica* species. Further work on this relevant area is required in order to understand *Fasciola* epidemiology and evolution as well as more effective means of parasite control.

Keywords: *Bubalus bubalis*, Diagnosis methods, Flukes, North East Argentina region.

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Diagnostic of endo and ecto parasites in buffaloes (*Bubalus bubalis*) during the four annual seasons, in two provinces in the Northeast of Argentina

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Introduction: The provinces of Corrientes and Chaco concentrate 45% of the exemplary bubalinos of the country. Argentina has 101.555 heads in 20 provinces (SENASA - 2016). The aim of this work is to determine the presence of endoparasites and ectoparasites in buffaloes. Materials and Methods: Four farms were selected of different categories of animals, in the four annual seasons in the provinces of Corrientes and Chaco. To carry out the analysis of internal parasites, it was taken the animals' fecal matter which was analyzed to diagnose copro-parasitologico using qualitative and quantitative techniques. For the detection of external parasites, it was carried out direct observation. Results: With regard to endoparasitos, the biggest prevalence of nematodes was observed in 51% of the animals, which were affected during spring. The highest percentage of *Fasciola* sp was 37.84% and coccidios was 32.43 % in winter. The percentages mentioned above were permanent during the research. It is worth mentioning the discover of *Strongyloides* sp, *Toxocara* sp., *Moniezia* sp. and *Capillaria* sp. in some animals. Regarding the ectoparasitosis, the permanent presence of *Haematopinus* sp was demonstrated as well as the presence of *Haematobia* sp. up to 80%. The "tick common of the bovine" was only present in 7%. The miasis was not present during the whole period of the study. Conclusion: the most important endo-parasitos that were observed in this research were the Nematodes, Coccidios spp, and *Fasciola* sp., during the whole year. *Strongyloides* sp. and *Moniezia* sp. were presented in bucerros in the most humid and cold months of the year. The ectoparasitos *Haematobia* sp. was presented in every season. *Haematopinus* sp. was observed in the 4 seasons of the year, but it was not observed in all the farms. *Rhipicephalus (B) microplus* was only observed in one of the farms. There were no cases of miasis during the study.

Keywords: Buffalos, Chaco, Corrientes, Nematodes, Prevalencia.

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Tuberculosis in meat water buffaloes (*Bubalus bubalis*) from Corrientes, Argentina: preliminary data

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The water buffalo is susceptible to Tuberculosis caused by *Mycobacterim bovis*. In Argentina is mandatory a National Program regulated by SENASA that establish the skin test with bovine PPD (purified protein derivative), sacrifice of positives and slaughterhouse epidemiologic surveillance, but buffaloes are not included. The aim of this work was to support incorporation of buffaloes into sanitary regulations and evaluate the results of Skin Testing in buffaloes. A total of 372 buffaloes from four flocks were skin-tested according to SENASA policies. This procedure took place in the caudal fold, by inoculating 0.1 ml of bovine-PPD and measurement after 72 hours. Differences among folds less than 3 mm were "negative", and higher were "positive". 47 animals were slaughtered and lymph nodes' and organs' samples with tuberculosis compatible lesions (TCL) were analyzed by PCR, histopathology and bacterial culture. From 372, 95.4% (n=355) were negatives, 46 of these were slaughtered and one presented TCL. On the other hand, 4.6% (n=17) were positives, one of them was slaughtered and seized by TCL. Histopathology and bacterial culture confirmed tuberculosis diagnosis. *M. bovis* isolation by PCR and spoligotyping reveled that Spoligotype 34 (SB0140) results in one of them and 12 (SB0120) in the other. The diagnosis measurement has been performed within the established basis of SENASA, regardless the ones suggested by Roxo (1998) were higher standards are recommended. There were correlativity between results of skin test and slaughterhouse inspection, disregarding the negative buffalo that presented TCL and could have been on anergy. None of the seized animals presented TBC symptoms before slaughter. The SB0140- Spoligotype was previously reported in buffaloes, while the SB120 was not. Skin testing based on SENASA policies proved to be valid in buffaloes for this investigation. There was correlativity bounded by the epidemiologic surveillance in slaughterhouses and skin testing.

Keywords: enzootic endemic, mycobacterium sp., tuberculinization.

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Infestation levels and distribution of *Haematopinus tuberculatus* in water buffaloes (*Bubalus bubalis*) in two farms of States Córdoba, Colombia

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Buffaloes are affected by ectoparasites such as flies, ticks, lice and mites, these besides discomfort can convey different infectious diseases. Lice of the family Haematopinidae are responsible of decreasing in production. The infested animal alters its natural resting habits and feeding will cause disquiet, severe itching and skin lesions. Nevertheless these ectoparasites are little studied because not generate mortality or noticeable economic losses. The aim of this study was to identify the genus and species of louse prevalent in water buffalo (*Bubalus bubalis*) naturally infected and their distribution in the body of the animal in two farms in pueblo Nuevo, State of Córdoba, Colombia. They were selected fifty bubalines Murrah breed in different production stages (lactation, raised) parasitized with lice. From each animal, age, skin lesions and degree of parasitism was recorded. The specimens (adults, nymphs and nits) were collected manually on each of the eight sites considered favorite infestation. They were put on vials containing alcohol 70% v/v. Later in laboratory were mounted in slides, analyzed in optic microscope and identified. All animals had high levels of infestation. The collected specimens were identified in its entirety as *Haematopinus tuberculatus* following key proposed by George Scott & Stojanovicch; Soulsby; Manning A. Price; Chaudhuri and Kumar. Most affected areas were tail (77.75%) and cheeks (22.25%). In the tail parasites was decreasing by evolutionary stage: eggs (47.5%), hatched eggs (21%), adults females (13.3%), adults males (5.7%), nymphs 1 (4%), nymphs 3 (4%) and nymphs 2 (3%). Similar percentages were presented on the cheeks and both tail and cheek was the largest infestation by eggs. *H. tuberculatus* was the most prevalent ectoparasite in buffaloes of different ages in the two farms studied and should be routinely considered their control may generate economic losses as well as its well-known vectorial capacity of various infectious diseases for these animals.

Keywords: ectoparasites, Haematopinidae, distribution, *Bubalus bubalis*.

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Evaluación de exámenes complementarios con orientación diagnóstica en búfalos positivos a tuberculosis por DPP

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La tuberculosis bovina es una enfermedad zoonótica de distribución mundial que representa grandes pérdidas económicas en la producción ganadera y es considerada una enfermedad de interés en los esquemas de prevención sanitaria en todos los países del mundo. Existen problemas en el diagnóstico de TBC en búfalos, que requieren del apoyo de otras pruebas diagnósticas complementarias. El objetivo de esta investigación fue realizar una evaluación comparativa de pruebas complementarias de orientación clínica en búfalos positivos y negativos a tuberculosis bovina mediante la prueba DPP. Fueron seleccionadas 13 búfalas de raza criolla provenientes de un solo hato ubicado en el trópico bajo de la zona norte de Colombia, todas las búfalas fueron sometidas a las pruebas de pliegue caudal y cervical comparativa para diagnóstico de DPP. Todos los animales fueron sangrados en la vena coccígea media, las muestras fueron procesadas para la realización de cuadro hemático y perfil sérico. Fueron evaluados 13 búfalas mestizas. 5 Búfalas (38,46%) fueron positivas a pliegue caudal y cervical comparativa. 8 búfalas (61,53%) eran negativas a cervical comparativa. Los eritrocitos, hematocrito, hemoglobina y volumen corpuscular medio presentaron diferencia estadísticamente significativa ($p \leq 0,05$) entre las búfalas sobre condicionadas positivas a la prueba de pliegue caudal y búfalas delgadas negativas. En la química sanguínea sérica para pruebas de funcionamiento renal, la medición de creatinina presentó una diferencia estadísticamente significativa ($p \leq 0,001$) entre las búfalas sobre condicionadas positivas y las búfalas negativas. Los valores del cuadro eritroide no presentan alteración en búfalas positivas a DPP. Los niveles altos de creatinina sérica en los búfalos positivos, relaciona este analito con la prueba positiva a DPP, esta situación podría interpretarse con un inicio de alteración de la función renal en pacientes positivos a DPP.

Keywords: tuberculosis, Buffaloes, diagnóstico TBC, DDP.

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Wide circulation of bubaline herpesvirus infection in Mediterranean Water Buffalo and implication in buffalo trade

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Both Bovine herpesvirus (BoHV-1) and Bubaline herpesvirus (BuHV-1) have been reported to cross the species barrier. Antibody seroconversion in glycoprotein E (gE) blocking enzyme-linked immunosorbent assay (ELISA) during BuHV-1 infection has been documented (1). BuHV-1 infection can lead to misdiagnosis of IBR, resulting in unjustified restrictions on buffalo trade. This has been demonstrated in France, where the only virus that could be responsible for IBR misdiagnosis is CpHV-1, since goats and cattle can be in close contact in some field situations (2) Recent diagnostic efforts have focused on the development and application of discriminatory tests to distinguish between infections with BoHV-1 and BuHV-1. We report the results of field application of BuHV-1 and BoHV-1 gE-blocking ELISA. Also, we present prevalence data of BuHV-1 in two regions of Italy (Piedmont and Campania) where Infectious Bovine Rhinotracheitis (IBR) control programs are in place. Sampling was carried out on 13 buffalo farms: 643 of 1089 (59.04%) serum samples reacted to ELISA (irrespective of whether BoHV-1 or BuHV-1 antigen), 555 (86.4%) of which were reactive to BuHV-1 and 76 (11.8%) showed absorbance values for both antigens and were classified as inconclusive. There was a statistically significant age-related difference in BuHV-1 infection rates but not in overall individual (46.99% vs. 58.24%) or herd prevalence (100% vs. 90%) of infection between the two regions. The low percentage of sera reactive to BoHV-1 (1.8%, 12/643) indicates that BuHV-1 may be the main circulating alphaherpesvirus infection in Mediterranean water buffalo in the two study areas. Since *Bubalus bubalis* is included in Directive 64/432/EEC (3) on animal health problems affecting intra-Community trade in bovine animals, diagnostic testing with nonspecific ELISA for BoHV-1 infection in buffalo may yield false-positive reactions. This scenario could lead to economic losses and hamper buffalo trade and movement, particularly for reproduction purposes.

Keywords: buffalo, Herpesviruses, BuHV-1, BoHV-1, ELISA.

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IFN- γ como marcador de la respuesta inmunitaria en búfalos de agua vacunados con RB51

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Ha sido ampliamente demostrado en bovinos, principalmente vacunos y en Búfalos de agua, que la cepa de *B. abortus* RB51 induce principalmente una inmunidad mediada por células, con la inducción de IFN-g y no de IL-4. *B. abortus* es un fuerte inductor de inmunidad mediada por células y la activación de macrófagos con IFN-g es un importante factor de control de la infección por *Brucella*. El IFN-g se expresa en las células de sangre periférica en bovinos vacunados, estimuladas "in vitro" con *B. abortus* cepa RB51. Este resultado expresado por varios Autores, confirma que la vacuna RB51 induce una respuesta celular con patrón de citocinas Th1. La prevalencia e incidencia de la brucelosis en la zona de estudio fue calculada por Card Test y confirmada por FP en suero. La presencia y concentración del IFN- γ se midió en sangre periférica de Búfalos, Bubillas y Bumautas, vacunadas o no, con varios protocolos de vacunación RB51, estimulada con Antígeno RB51, utilizando un ELISA de captura para IFN- γ bovino. La prevalencia de brucelosis supero el 20% en la zona. La respuesta IFN- γ en Bubillas fue significativamente mayor al de Búfalos y Bumautas mostrando además altas concentraciones de IFN-g especialmente 30 días después de revacunadas con RB51. Los protocolos reforzados de vacunación RB51 respondieron mejor que los oficiales. Las Bumautas respondieron muy poco a pesar de haber recibido vacunación completa. Se concluye que el IFN-g puede ser utilizado para monitorear la calidad de la respuesta celular de Búfalos adultas frente a la vacuna RB51.

Palabras clave: búfalos, RB51, *B. abortus*, IFN- γ , Card Test, FP.

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Novel approaches for diagnosis of bacterial agents responsible for abortion in water buffalo through metagenomic analysis

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Reproductive disorders of bacterial origin represent an important problem in water buffalo herds. During past decades several studies identified and characterized microorganisms responsible for abortion by conventional microbiological techniques including isolation and biochemical characterization. The advent of molecular techniques such as PCR detection of single genes, first, followed by filogenetic studies based on 16S rRNA characterization, through molecular cloning, denaturing gradient gel electrophoresis (DGGE) or terminal restriction fragment length polymorphism analysis (TRFLP) allowed much more informative data. These studies contributed to identify as most frequent causes of abortion in water buffalo *N. caninum*, *Chlamydia* spp., *Brucella* spp., *Coxiella*, *Leptospira* spp., BHV1, BVDV. The recent advances in molecular biology permitted the diffusion of Next-Generation Sequencing (NGS) platforms, allowing the molecular detection and identification of non culturable and non viable microorganisms. Indeed metagenomics applies a suite of genomic technologies and bioinformatics tools to directly access the genetic content of entire communities of organisms. The field of metagenomics has been responsible for substantial advances in microbial ecology, evolution, and diversity over the past 10 years. The present study illustrates a comparative analysis between abortion diagnoses in 16 water buffalo fetuses achieved by classical microbiological methods and metagenomic analyses.

Key words: 16S, NGS, pathogens.

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Q fever: study of environmental distribution of *coxiella* in water buffalo farms of the Campania Region

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Q fever is a zoonotic infection caused by *Coxiella burnetii*, a pathogen worldwide spread. Ruminants and pets represent the most important reservoirs of the infection, although wild species can have a role in the transmission of *Coxiella* to animals and humans. Recent outbreaks occurred in the Netherlands indicate that Q fever is a re-emerging disease. Indeed the EU requested further scientific studies and risk assessment, for a better understanding of prevalence and distribution of Q fever in Europe. In this context, the aims of this study were to investigate the seroprevalence of *C. burnetii* in water buffalo farms characterized by reproductive disorders and evaluate the presence of this pathogen in farming environments and wildlife by molecular detection of *Coxiella* DNA.

Keywords: *C. burnetii*, ELISA, epidemiological surveillance.

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Characterization of clinical-pathological picture caused by compressive injuries in the Central Nervous System (CNS) of buffaloes in the state of Pará - Brazil

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There are several etiological agents that promote changes in the central nervous system (CNS) of farm animals. However, other causes of changes in the CNS are compression injuries caused by abscesses, neoplasms, and fractures of vertebrae of the spine. The clinical signs shown by the animals are variable, being linked directly to the injured site, degree of spinal cord compression and the involvement of the spinal anatomical tract. This study aimed at characterizing the clinical-pathological picture in buffaloes with compressive injuries in the CNS. A survey of all clinical care records of buffaloes diagnosed with nerve changes assisted by veterinarians from the Veterinary Diagnostic Center (CEDIVET) belonging to the Federal University of Pará, Brazil, in the period January 1998 to December 2014 was carried out. We evaluated the epidemiological data, clinical signs and necropsy findings. Clinically diagnosed cases comprised six buffaloes. Of affected buffaloes, four were male and two were female, aged between two and ten years, of Murrah breed, from properties located in city Castanhal, Santo Antonio do Taua and Ilha do Marajo, Pará, Brazil. In none of the properties that create buffaloes, mineral supplementation was performed. Animals affected by spinal cord injuries showed, in general, paralysis, inability to get up and keep on station, postural changes, hyperreflexia in the extremities and loss of surface sensitivity in areas related to the injury. Of the six buffaloes, five had injuries in the spinal cord segment between T3-L3 and one between L4-S2. The diagnosis of all cases was given taking into account the background, clinical signs and necropsy findings. We concluded in this study that the clinical signs associated with necropsy findings proved sufficient to characterize the clinical picture and locate the affected segments of the CNS.

Keywords: farm animals, buffalo, spinal cord injuries, Neurological signs.

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Risk factors for ectoparasites in buffalo from Santarém, Amazon, Brazil

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Ectoparasites are responsible for economic losses in buffalo production, being the lice *Haematopinus tuberculatus* the most important. In recent study at Rio de Janeiro more than 80% of farms showed buffaloes parasitized by ticks; regarding the Brazilian Amazon, there is no recent report of tick's in buffalo. Thus, the aim of this study was to determine the occurrence and the risk factors of ectoparasites in buffaloes raised at Santarém. Forty buffalo farms were visited (representing 16.3% of total farms), 32 in the mainland and 8 in the wetlands. In each farm the number of buffalo sampled was according to heard size. The selected animals were examined for ectoparasites, which, if found, were stored in plastics tubes with ethanol for identification at species level throughout specific keys. Risk factors considered were the farm location (mainland/wetland), gender, breed, usage, age, presence of wild animals or bushes. Of the 40 farms, 505 buffaloes were sampled, 201 males and 304 females; 138 calves, 138 steers and 229 adults. Ticks were found in 39 buffaloes (7.72%), identified as *Rhipicephalus Boophilus micropulus* (1 nymph and 45 adults ticks) infesting 22 and *Amblyomma cajennense* (36 adults) infesting 17 buffaloes. The lice *Haematopinus tuberculatus* was found in 68 buffaloes (13.46%); 5 animals showed mixed infestation. At farm level, the occurrence of ticks was 30% and for lice 50%. At animal level, the risk factors associated with tick infestation were: farm location ($p=0.006$), age group ($p=0.005$) and presence of wild animals ($p=0.014$). For lice, the farm location ($p=0.006$) and age group ($p=0.018$) were associated with infestation. Buffalo calves were more infested by ticks than older animals; inversely, they were less infested by lice. Buffalo raised in the wetland showed lower infestation of lice and ticks than animals kept in mainland.

Keywords: infestation, lice, ticks, wetland.

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Conservation of buffalo (*Bubalus bubalis*) whole blood in CPDA-1 and CPD/SAG-M BAGS

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The whole blood transfusion in farm animals objective the restoration of the capacity of transport and diffusion of oxygen to the tissues in cases of significant reduction in hemoglobin, as in severe anemia or acute blood loss. Thus, it was aimed to evaluate hematological, blood gas and biochemical alterations and transfusion viability of buffalo whole blood stored in CPDA-1 and CPD/SAG-M bags for 42 days. We used ten adult, male, healthy, mixed-breed buffalo, from each 900 g of whole blood were collected and subsequently stored in CPDA-1, and CPD/SAG-M bags (450 g in each bag) and kept under refrigeration at 2 °C to 6 °C for 42 days. Whole blood stored in plastic bags were evaluated at seven moments: D0 (immediately after collection of blood), D7, D14, D21, D28, D35 and D42, after 7, 14, 21, 28, 35 and 42 days after collection, respectively. At those moments, hematological, blood gas, biochemical and microbiological analysis were performed. Increases were observed for partial pressure of oxygen and carbon dioxide, lactate and potassium whereas red blood count, pH and sodium bicarbonate and glucose concentration decreased over time. Total hemoglobin concentration and packed cell volume remained stable. At the end of experiment, the amount of erythrocytes was 80% of the initial count and the degree of hemolysis was 10.4 and 9.01% for CPDA-1 and CPD/SAG-M bags, respectively. Buffalo whole blood stored in CPDA-1 and CPD/SAG-M plastic bags underwent hematological, blood gas and biochemical alterations, however remaining viable for transfusion when stored for up to 42 days at temperatures of 2 to 6°C. In general, there were no clinical importance differences in the conservation of whole blood buffalo between the two types of bags (CPDA-1 and CPD/SAG-M) evaluated, being both suitable for use in this species.

Keywords: buffalo, conservation, hematology, transfusion.

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Identificación molecular de *Leptospira spp.* patógenas en cultivos provenientes de fetos bovinos abortados

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Las espiroquetas constituyen un grupo de bacterias únicas en términos de su evolución e incluyen patógenos médicamente importantes como el agente causal de la enfermedad de Lyme, sífilis y leptospirosis. El género *Leptospira* pertenece al phylum Spirochaetes, clase Spirochaetes, orden Spirochaetales, familia Leptospiraceae y comprende especies saprofitas y patógenas que conforma un grupo evolutivo único. La rama más ancestral del género está compuesta por las leptospirosas saprofitas que incluyen *L. biflexa*, otro subgrupo incluye las especies patógenas con 7 especies como *L. interrogans*, *L. borgpetersenii*. Otra rama evolutiva comprende el grupo de las leptospirosas intermedias, cuya patogenicidad no está clara. Se recibieron 23 fetos en el Centro Diagnóstico Veterinario Rosaura Pérez Gil, ubicado en la ciudad de Araure, estado Portuguesa, Venezuela, en el periodo de abril 2006 a mayo 2014, provenientes de 17 fincas, ubicadas en los estados Apure (2 fincas), Aragua (1 finca), Barinas (1 finca), Lara (1 finca), MÃ©rida (1 finca), Monagas (2 fincas), Portuguesa (6 fincas), Trujillo (1 finca) y Zulia (2 fincas); de estos 23 fetos 7 pertenecían a la especie *Bos indicus*, 7 a *Bos taurus* y 9 a *Bubalus bubalis*. Los fetos fueron envueltos en bolsas plásticas en la finca donde se capturaron, ésta fue cerrada herméticamente, refrigerados con hielo y enviados al laboratorio en cava de anime. Al recibirlos se evaluó la integridad del feto, que no presentara mordeduras de animales, signos de autólisis post mortem ni olor fétido que indicara descomposición. Las 3 bacterinas comerciales Leptoferm-5[®], Lepto Shield[®] y Vira Shield[®] y las cepas de referencia *Leptospira pomona pomona* y *Leptospira hadjopratjino* amplificaron los dos set de marcadores utilizados en este estudio (G1-G2 e Internal 1- Internal 2) generando bandas de aproximadamente 285 pb para G1-G2 y 497 pb para Internal 1- Internal 2. Se concluye que la técnica de Reacción en Cadena de la Polimerasa (PCR) con los marcadores G1-G2 e Internal 1-Internal 2 y el método de extracción de ADN Fenol/Cloroformo-Alcohol Isoamílico, es útil para el diagnóstico de *Leptospira spp.* patógenas en fluidos de fetos abortados de las especies *Bos indicus*, *Bos taurus*, y *Bubalus bubalis*. Los fluidos a partir de los cuales se logró una mayor detección de *Leptospira* con el marcador G1-G2 son: fluidos renal y materno-fetal, y para el marcador Internal 1- Internal 2 son: fluidos abdominal y ocular. El método de extracción de ADN mediante el kit comercial Wizard[®] Genomic DNA Purification mejora la capacidad de la PCR para detectar *Leptospira spp.* patógenas con el marcador G1-G2 y el marcador Internal1-Internal 2. Se sugiere ampliar los estudios de leptospirosis en fetos abortados con métodos moleculares de diagnóstico.

Palabras clave: fluidos fetales, *Leptospiras spp.* patógenas, PCR.

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Evaluation of a commercial Elisa kit using buffalo (*Bubalus bubalis*) serum pools as a monitoring tool for bovine brucellosis at dairy farm in the south lake, Maracaibo, Venezuela

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Brucellosis is one of the most important bacterial zoonosis in cattle and water buffalo (*Bubalus bubalis* var. *Bubalis*), the disease is characterized by abortions in the last third of the gestación. Because of this it was proposed to evaluate the performance of commercial kit for Indirect Elisa (*IDEXX® Brucellosis Serum Ab Test*) using the format mixtures or serum pools as monitoring test bovine brucellosis, against fluorescence polarized assay (FPA) and bengal rose (BR). A total of 3280 buffalo sera from the southern region of Lake Maracaibo, were referred for screening bovine brucellosis and subjected to Bengal Rose, FPA and indirect ELISA format mixed or pooled sera. The Elisa-I obtained a negative 78.26% and 21.04% pools positive pools. FPA with a total of 29 (0.88%) of the animals were seropositive for *Brucella* sp., and 3251 (99.11%) were seronegative. The estimated Elisa-I pooled sera against FPA area under the ROC curve was 0.928 and 0.905 BR. Kappa values Elisa-I pool against FPA with the cutoff for S / P of 35% and 30% are 0.530 and 0.518, sensitivity was 41.7% and 91.3% and specificity of 98.9% and 89.5% respectively. The format ELISA serum pools of buffalo sera, is a good choice for monitoring bovine brucellosis, establishing points based on epidemiological conditions of the study area cut, followed increase the cutoff to a value of S / P 30%, to improve the values of Se, Sp, and Kappa.

Keywords: bovine brucellosis, water buffalo, *Brucella* sp.

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Uso de las técnicas Flujo Lateral y ELISA captura de Antígeno para el virus de la Diarrea Viral Bovina (BVDV) en establecimientos bufalinos de Venezuela

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La Diarrea Viral Bovina es una enfermedad viral de los bovinos producida por el virus de Diarrea Viral Bovina (BVDV) provoca fallas reproductivas, hidrocefalia e hipoplasia cerebelar, el virus ha sido aislado en fluidos fetales, pero se conoce poco sobre su rol como agente causal de abortos. El propósito de esta investigación fue capturar antígeno viral de BVDV captura de antígeno BVDV en muestras de tejido, mediante el uso de las técnicas de flujo lateral y ELISA. Se analizaron de 695 muestras de tejido de búfalos lactantes procedentes de 5 establecimientos de Venezuela, resultando todas las muestras negativas a la técnica flujo lateral. Con la técnica ELISA captura de antígeno BVDV, se logró demostrar una animal con infección transitoria al virus BVDV en el establecimiento C y una alta sospecha de presencia de actividad viral en los establecimientos B y D por lo que se sugiere ampliar los estudios en la especie bufalina para poder afinar las medidas de prevención y control del virus de las Diarrea Viral Bovina en establecimientos bufalinos de Venezuela.

Palabras clave: *Virus Diarrea Viral Bovina, ELISA, tejido, antígeno, búfalo.*

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Evaluation of comparative effectiveness of Fipronil 1% and Deltamethrin 5% topically applied against lice in water buffalo (*Bubalus bubalis*) naturally infested

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Introduction: The sucking louse *Haematopinus tuberculatus* (Burmeister 1839) is a most prevalent ectoparasite in buffaloes. The infested animal alters its natural resting habits and feeding will cause disquiet, severe itching and skin lesions. A trial was conducted on buffaloes infested by these lice to assess the efficacy and safety of two active substances at recommended doses for cattle by commercial houses. **Materials and Methods:** In a commercial farm in -Pueblo Nuevo, Córdoba - Colombia, 24 bubaline adult females, breed Murrah, naturally infested by lice, with approximate age of 5 years were selected. These were divided into two homogeneous groups of 12 animals. A group was treated topically with Fipronil 1% (CARVAL - of Colombia) single dose of 1 ml of product per 10 kg of body weight, applied "Pour on" between the cross and the base of the tail. The second group was treated with Deltamethrin (5%, BIOARA S.A.) at a dilution of 0.1%, applied in a single bath with pump spray back equal to 4 liters of dilution per animal. Each treated buffaloes was performed from day 1 to day 56 post-treatment (once a week). **Results:** Both products were well tolerated by all animals as no adverse reactions occurred. From day 30 in animals treated with fipronil reinfestations only they reached 11%, while the group treated with deltamethrin were almost complete reinfestations reaching 98%. Fipronil showed efficiency near 100% (97 %) from day 37 to day 56 post-treatment (end of study). **Conclusions:** The results of this trial suggest that both products are effective, safe, and user-friendly compound suitable for treatment of buffaloes with natural louse infestations. However Fipronil is potentially more effective because it reduces the amount of baths to perform in infected animals.

Keywords: ectoparasites, pediculosis, buffalo, treatment, effectiveness.

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Evaluación de dos técnicas de diagnóstico en un rebaño bufalino infectado con *Mycobacterium bovis*

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Bovine tuberculosis is an infectious disease whose causative agent is *Mycobacterium bovis* (*M bovis*), worldwide distribution, zoonotic widely distributed in cattle including buffalo. In buffalo must be considered within the epidemiology of the disease and habitat of these physiological and anatomical conditions, conditions that make different the approach in the field diagnosis and the mechanisms described in the pathogenesis of the disease. For this reason the comparative tuberculin test and the ELISA test in conjunction with the post-mortem evaluation and detect Bacteriological to achieve the highest number of infected animals culture was used. A herd of 700 buffaloes from different age groups was tested, comparative tuberculin test in Table neck was applied to the herd. Animals tested negative for comparative tuberculin test a test were applied ELISA antibody against M bovis. The reactants to ppd bovis and TBC ELISA positive animals were euthanized animals and subjected to post-mortem inspection, Tissue samples were taken and transported to the laboratory for studies BK and microbiological culture and identification of the pathogen. 72 ppd bovine reactants in comparative tuberculin skin test and 4 shall not reaccionantes to comparative tuberculin test but positive ELISA test animals. The 76 animals were brought to slaughter and subjected to post mortem where macroscopically compatible with tuberculosis lesions showed inspection. Tissue samples were taken and processed being observed to Ziehl Neelsen acid-fast bacilli and identification of M bovis through bacteriological culture. Tuberculosis affecting the buffalo herd in the study was mainly digestive and not respiratory, not all infected animals react to the comparative tuberculin, test thus requiring use other diagnostic tools, the habitat of farms can increase the level of infection within the herd.

Keywords: bovine tuberculosis, comparative tuberculin skin test, ELISA, Buffaloes.

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