**In ovo** prebiotic administration – preliminary results

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**ABSTRACT**

*In ovo* technology is based on direct injection of different substances to the chicken embryo during embryogenesis. This technology has become an alternative choice of vaccination of broiler chicks against diseases; other applications are stimulation of chicken embryogenesis, transgenic chicken production, teratogenic effect testing, and determination of gender in a fertile poultry egg. In recent years, we developed a method to inject the raffinose family oligosaccharides (RFOs) in the air space of egg, and we tested the prebiotic effects on the chicken. Up to now these oligosaccharides were considered as antinutritional factors because they are not hydrolyzed by mucosal enzymes in the small intestine of monogastric animals. The raw materials of RFOs are legumes seeds – mainly lupin pea and lentil. In order to obtain high purity RFOs preparations we elaborated a simple method of their isolation and purification. The main steps of this method are: extraction with alcohol, inactivation of galactosidases, absorption and ion exchange chromatography. This method allows to obtain about 50 g (96% purity) of preparation from 1 kg of lupin seeds. We showed that our preparation, in dependence on source, holds different sugar composition. They are not toxic (acute toxicity, expressed as LD₅₀ is above 6 g/kg body weight) and may have effect on the immune system. To study the prebiotic effect of *in ovo* RFOs administration two different trials were carried out. The objective of the first, realized in laboratory condition, was to determine this effect on the chicken hatchability and on the intestinal microflora of chicken. RFOs were injected into the air space of 100 eggs from each group (at 12 day of incubation) with 0.2 mL of Ringer water solution containing the following doses of preparations: 0.0; 0.69; 3.43; 6.87 mg/egg. The hatchability of eggs and amount of intestine bifidobacteria, E. coli and anaerobes presence in 1 g of sample of 2 days old chicken, were registered. RFOs in ovo administration had no effect on hatchability but increased significantly \( P \leq 0.01 \) the beneficial intestinal bifidobacteria in birds. The aim of the second trial, realized in the technical scale, was to determine the effect of *in ovo* prebiotic administration on the hatchability and body weight of chickens. Automatically system of RFOs injection (about 30,000 injections per hour) was constructed. The hatchability of injected eggs was 90.72% (36,470 chickens from 40,199 eggs treated at 12th day of incubation) and was significantly higher \( P \leq 0.01 \) compared to untreated eggs – 89.0% (53,440 chickens from 60,046 eggs at 12th day of incubation). The body weight of 100 randomly chosen chickens was 403.8 g versus 396.9 g and 816.4 g versus 776.1 g in 2nd and 3rd week of age, in RFOs injected and uninjected group, respectively. The difference in the 3rd week between treated and untreated chickens was statistically significant \( P \leq 0.05 \). In conclusion, the results of these two studies demonstrated that *in ovo* prebiotic administration is an efficacious and convenient method of broiler production.
Phenotypic characterization of the Italian chicken breed Mericanel della Brianza

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ABSTRACT

This study was aimed to characterize the phenotypic features in a small population of the Italian chicken breed Mericanel della Brianza (white). Fifty one birds have been acquired from a local fancy breeder in three different reproductive seasons, therefore birds’ age ranged from 5.5 to 30 months. Twenty two cocks and 29 hens were measured and weighted. The following phenotypic traits have been recorded: plumage colour, skin colour, eye colour, earlobe colour, tibia tarsus colour, comb type. Body weight, keel length, body length, tibia tarsus length and diameter have been measured. All the birds had white plumage, simple red comb and orange eye. The majority of birds had yellow skin, yellow tibia tarsus and red earlobe. Phenotypic quantitative traits showed a great variability. The breed is characterized by sexual dimorphism; males had heavier body weight and larger body size compared to females. Mean body weight recorded in males was 961±161 g and in females was 607±151 g. The age had a significant effect on all the quantitative traits in both sexes. Phenotypic qualitative features agreed with the Federazione Italiana Associazioni Avicole (F.I.A.V.) breed standard published in 1996, in contrast some quantitative characteristics measured in the present population were different compared to the breed standard. The results of this trial stress the importance of evaluating other characteristics to better define Mericanel della Brianza features and to program breeding strategies to meet the standard characteristics.
Correlation between different physiological parameters (hormonal, oxidative, immune) in Italian poultry breeds

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Abstract

Animal welfare is a “state” that encompasses many complex aspects of the animals and includes biological, psychological and behavioural components. Physiological variables, that include hormone levels such as cortisol or corticosterone or immune status, are frequently used as reliable indicators of the welfare status. The aim of this preliminary study was to better understanding dynamics and interaction between hormonal status, innate immunity and oxidative stress of some Italian poultry breeds. Three hundred sixty chickens were used and in particular: 120 of medium growth rhythm (60 Robusta maculata and 60 Italian Necked Neck); 240 of slow growth rhythm (60 Valdarnese Bianca, 60 Bionda piemontese, 60 Ancona and 60 Livorno). All animals were placed in a closed space with high density (15 chickens/m²). Before slaughtering (82 days of age), blood samples of all birds were obtained by vein puncture (ulnar vein) for evaluation of the following variables: plasma corticosterone and glucose, serum lysozyme, bactericidal activity, haemolytic complement, acute-phase proteins, free oxygen radicals and derivatives and total antioxidant capacity. Plasma corticosterone and glucose were positively correlated (P<0.05) revealing metabolic changes during physiological stress in chickens. Lysozyme was negatively correlated with haemolytic complement (P<0.007) and bactericidal activity (P<0.003) probably due to the presence of sub-inflammatory processes which enhance the release of lysozyme by neutrophiles and macrophages and reduce the free complement which is mainly found in immuno-complexes and the bactericidal activity. Haemolytic complement and bactericidal activity were positively correlated (P<0.0001), confirming their immune function as early defence barriers. The same explanation could be hypothesized for the positive correlation (P<0.0001) between bactericidal activity and free radicals released by leucocytes during inflammatory processes. A positive correlation (not significant) between lysozyme and total antioxidant capacity was found, due to the body’s need to compensate for the higher free radicals level. This hypothesis could be confirmed by the positive correlations between free radicals and acute-phase proteins (P<0.002) and corticosterone (P<0.0001). Moreover antioxidant capacity was negatively correlated with acute-phase proteins (P<0.02), haemolytic complement (P<0.003) and bactericidal activity (P<0.01) and free radicals (P<0.04). Analysing differences between growth rhythms, slow growing chicks showed significant lower values of bactericidal activity (42.1 vs. 56.0%), haemolytic complement (36.8 vs. 44.8 CH50/150 μl), acute-phase proteins (0.40 vs. 0.52 mg/ml) and ROMs (5.70 vs. 6.01 mmol H₂O₂). These results would imply that chickens (especially those at medium growing rhythm) reared at high density have some difficulty in balancing environmental stimuli, therefore provoking an increase in non-specific body defences. More detailed investigations about acute phase proteins could clarify homeostasis of innate immune status, their actions and presence, being strongly correlated with inflammatory response and stress.
Structural changes of turkey spermatozoa after cryopreservation with the pellets method: a Scanning Electron Microscopy study

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ABSTRACT

A Scanning Electron Microscopy (SEM) study was conduct in order to determine and localize cells cryoinjuries in turkey spermatozoa following freezing/thawing with pellet method, a promising cryopreservation technique consisting in freezing and packaging samples as pellets produced in liquid nitrogen. Six pooled semen samples were collected via dorsum-abdominal massage technique from 20 Hybrid Large White turkey toms. Samples were diluted 1:4 with the Tseltulin extender (Na Glutamate 128 mM, K₂HPO₄ 20 mM, glucose 44.4 mM, inositol 11.1 mM, Mg acetate 7 mM, glycine 13.3 mM, glutamic acid 7.68 mM, pH 6.65), cooled, added with 8% of dimethylacetamide as cryoprotector and aliquots of 80 μL were directly plunged into a liquid nitrogen bath to form frozen pellets. Thawing was quickly performed at 75°C. Aliquots of 0.1 mL of both fresh and frozen-thawed semen were fixed in 1.9 mL of a 3% gluteraldehyde solution, dehydrated in ethanol, critical-point dried, evaporated with gold and examined with SEM (Zeiss DSM 940-A) for a morphological assessment at X10000 magnification. At least 200 spermatozoa per sample were observed and, on the basis of the structure displayed, were classified as undamaged (integral structure) or damaged (altered structure) spermatozoa paying attention to observe the overall plasmalemma and acrosome membrane integrity appearance. Results were expressed as mean percentages ±SD. Data were compared using a t test (SPSS 14.0, 2005 version). Freezing-thawing process strongly affected the structural integrity of turkey sperm cells, passing from 9±4% of damaged cells in unfrozen samples to 80±9% in frozen-thawed ones (P<0.001). In unfrozen spermatozoa studied by SEM, both plasmalemma and acrosome appeared to be intact, except for a few cases of slight membrane destructuration, principally at the level of the acrosome. Conversely, in frozen-thawed semen, very few sperm cells appeared to be intact, whereas the large number of spermatozoa were damaged, displaying different degrees of membranes destructuration. The less damaged cells showed minor changes in the plasmalemma and acrosomal membrane. The majority of damaged cells showed the outer part of the acrosomal membrane extensively disintegrated and the plasmalemma partially discharged, particularly at the level of the midpiece, whereas the higher damaged spermatozoa showed extensive loss of plasmalemma and complete absence of the acrosomal membrane. In conclusion, we showed by SEM a high loss in structural integrity of turkey spermatozoa following pellet method freezing-thawing process: spermatozoa displayed cryoinjuries both at the level of acrosomal region and head, midpiece and tail membrane, with the higher alterations occurring at the level of the midpiece, site where are localized mitochondria, enabling so the motility to the functional spermatozoa. Therefore further researches are needed to improve cryopreservation methods for turkey spermatozoa.
Assessment of microbial content of turkey (Meleagris gallopavo) semen after cryopreservation by the pellet method

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ABSTRACT

Avian anatomy is such that semen is exposed to the cloacal contents, providing ample opportunity for its contamination by commensal and/or pathogenous bacteria coming from the gastroenteric tract. This topic is particularly important in turkey breeder industry, where reproduction is relied solely by artificial insemination, because of the differences in size between sexes caused by advanced genetic selection. So bacteria could be spread from semen throughout entire flocks via artificial insemination. Pathogenic bacteria as Campylobacter spp. and Salmonella spp. may cause illness to humans through consumption of contaminated turkey products, whereas commensal microorganisms such as coliforms, Enterobacteriaceae and Enterococci compete with spermatozoa for the nutrients present in seminal plasma and/or in extenders, decreasing the semen quality and lowering the fertility of hens. Cryopreservation of turkey semen could be beneficial to the turkey breeder industry extending its use in time and space, but little attention has been payed on the possibility to vehiculate bacteria via cryopreserved semen. For this purpose, a study was conduct to assess the level of microbial contamination in fresh turkey semen and the microbial content after semen cryopreservation by the “pellet method”. Pooled semen samples were diluted 1:4, cooled, added with 8% of dimethylacetamide (DMA) as cryoprotectant and aliquots of 80 μL were directly plunged into liquid nitrogen to form frozen pellets. Thawing was performed at 75°C. logarithm of colony forming units per mL (log CFU mL⁻¹) of mesophilic viable counts, total and faecal coliforms, Enterobacteriaceae, Enterococci, Salmonella spp. and Campylobacter spp. were investigated on fresh and thawed samples plated and cultured according to the specific growth conditions of the microorganisms investigated. Fresh semen was highly contaminated by commensal bacteria and the cryopreservation process reduced microrganisms just of about 1 log CFU mL⁻¹, passing from 5.22±0.66 and 4.35±0.79 to 4.32±0.58 and 3.32±0.4 for mesophilic viable counts and total coliforms (P<0.05), and from 4.07±0.22, 4.21±0.53 and 4.26±0.08 to 3.18±0.52, 3.19±0.37 and 3.67±0.08 in faecal coliforms, Enterobacteriaceae and Enterococci (P<0.01), respectively. Conversely neither Campylobacter spp. nor Salmonella spp. were found in both fresh and cryopreserved samples. In conclusion, the pathogenous bacteria investigated were not found in turkey semen, however higher rates of commensal bacteria were recovered in fresh turkey semen and the cryopreservation process contributed to a slight reduction of these bacteria.
Effect of the pellet cryopreservation method on the post-thaw recovery of *Salmonella* spp. experimentally inoculated in turkey (*Meleagris gallopavo*) semen

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**ABSTRACT**

Contamination of poultry and turkey flocks by human enteric pathogens such as *Salmonella* spp. is a major concern to the poultry industry: bacterial contamination of animals can easily lead to contaminated eggs and meat products, causing human foodborne illness. Recently, attention has been payed in investigate the possibility of animal contamination through challenged semen, particularly in turkeys, where the advance in genetic selection has produced heavy commercial lines that cannot mate naturally and artificial insemination (AI) is necessary for reproduction: it has been showed that if fresh turkey semen is contaminated by *Salmonella* spp., the possibility to spread bacteria via artificial insemination to hens, eggs and chicks exist. An other unsolved problematic for turkey breeder industry is that despite to the dependence of turkey industry for the AI, methods for successful cryopreserve turkey semen have still not be found. In the last few years researches are focused on study of a simpler method of avian sperm cryopreservation, consisting in freezing and packaging samples as pellets produced directly in liquid nitrogen (pellet method), with some encouraging results. However, to the best of our knowledge, no data on the effect of freezing/thawing process of contaminated semen on post-thaw *Salmonella* spp. concentration are available. The objective of present work was to evaluate the possibility of *Salmonella* spp. transmission through the use of cryopreserved semen, evaluating the post-thaw survey of three emerging serovar of *Salmonella* spp. experimentally inoculated in turkey semen. Six pooled semen samples were divided into three subsamples and challenged with 7.8±0.2 logarithm of colony forming units per mL (log CFU·mL⁻¹) of *Salmonella* Liverpool, *Salmonella* Montevideo and *Salmonella* Braenderup, respectively. Samples were cooled, added with 8% of dimethylacetamide as cryopreserver and aliquots of 80 μL were directly plunged into a liquid nitrogen bath to form frozen pellets. Thawing was performed at 75°C. *Salmonella* spp. colonies were enumerated on serially diluted samples plated on HEKTOEN agar plates after incubation overnight at 37°C. Both *Salmonella* Liverpool, *Salmonella* Montevideo and *Salmonella* Braenderup colonies were recovered in thawed samples and showed a significant reduction of 2.03, 3.08 and 2.72 log CFU mL⁻¹ respectively, with respect to the fresh semen (P<0.001). Our study shows that *Salmonella* spp. may be transmitted via cryopreserved semen to flocks and pellets cryopreservation technique can only reduce the amount of these pathogen bacteria in thawed semen.
Evaluation of welfare in Italian poultry breeds based on genetic parameters

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ABSTRACT

Over the last years, control of animal welfare has become a major issue and even a point of contention in aviculture together with the sustainable development of breeding. In fact, in highly intensive livestock farming animals not always express their natural behaviour and they get a stress that reduces the performances both qualitatively and quantitatively. Consequently, there is an increased need to define specific stress markers. The measurement of plasma corticosterone level is widespread in aviculture. Alterations in hypothalamus-pituitary-adrenal (HPA) axis secretion are associated with an altered animal welfare. However, because of the great number and variability of the stressors in avian breeding, other indicators should be also studied. Also the molecular genetics studies allow to evaluate the stress condition. Mainly in medical field has been pointed out the existence of strong genetic component in the stress predisposition. This is particularly true for the genotype of loci involved with the glucocorticoid hormones function. In human was observed that the regulation of the glucocorticoid receptor gene NR31C (Nuclear Receptor subfamily 3, group C, member 1) expression is involved in the control of depressive syndromes. The aim of the work was to study the response of three different Italian poultry breeds (Ancona, Livorno and Naked neck) to a number of stressors by taking into account genetic markers and, in particular, to define glucocorticoid receptor gene expression in these animals. At this purpose, 180 animals (1 day old) of the three breeds before mentioned (60 for each breed) were raised in stressful conditions: the stress agent was represented by the rearing density (15 heads/m² or 30 kg/m²). In order to get an indicator of the level of stress suffered by animals, was determined the level of plasma corticosterone at the age of 40 and 80 days. At 81 days all the chickens were slaughtered and samples of liver were collected from each bird, immediately frozen in liquid nitrogen and stored at -80°C. From the samples of the liver, total RNA was extracted and utilized for the synthesis of the cDNA necessary for the reactions of real time PCR that quantify the absolute level of expression of the NR31C gene. The reaction was conducted using a pair of specific primers and TaqMan probe. The rate of corticosterone in the blood was then correlated with the levels of NR31C expression. The study is in progress but this stress indicator, for its genetic derivation, can be considered suitable for a scheme of divergent selection in order to create chicken genetic lines well adapted to different breeding systems. Particularly, for intensive breeding, can be constituted genetic types characterized by a low level of corticosterone and high expression of GR, while, for plain-air rural production, can be useful genetic types with opposite characteristics.
Technological characteristics of broiler breast meat processing in Sicily (Italy)

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ABSTRACT

Colour and pH poultry meat are critical food quality attributes. Colour is important for both the consumer’s initial selection of a raw meat product in the marketplace and for the consumer’s final evaluation and ultimate acceptance of the cooked product upon consumption. For this reason, it has therefore been suggested that Lightness (L*) values can be as an indicator of poultry breast meat quality for further processing and for evaluating the incidence of the pale, soft, and exudative (PSE)-like condition in poultry. The objective of this study was to evaluate some technological characteristics of broiler breast meat processing in Sicily (Italy). The study was carried out on 18,000 Ross 508 one-day-old broiler chickens (mean weight 42 g). In total, 6 floor pens (surface area 1800m²) were used, each containing 15,000 male and 15,000 female broilers. The birds were given ad libitum access to feed and water. A lighting schedule of 20L:4D was imposed throughout the experimental period. Ambient temperature was gradually decreased from 32°C on d 1 to 20°C at the end of the experiment. The trial was carried out from January 2006 to April 2007 at the AVIMECC S.p.A. slaughterhouse (Modica, RG - Italy). In each pen, 120 birds were randomly selected at 42 d (females) and at 52 d (males), therefore, slaughtered. At 24h post-mortem the breast muscles (Pectoralis major) were removed and pH24, Colour (CIE values L*, a*, b*), Hue, Chroma, cooking loss of ground meat and Warner-Bratzler Shear force (WBS) were measured. Pectoralis m. muscles were selected based on L* and pH24 as being Dark (L*<50 and pH≥6.0), Normal (50≤L*≤56) and Pale (L*>56 and pH<5.8). Data were subjected to ANOVA (proc. GLM by SAS, 2001) considering the variable: sex. No significant differences were observed for L* (females 56.33 vs. males 55.87; P=0.08), redness (a*) (females 1.59 vs. males 1.81; P=0.08), Chroma (females 2.47 vs. males 2.22; P=0.26), WBS (females 0.92 Kg/f vs. males 0.94 Kg/f; P=0.28) and pH24 (females 5.85 vs. males 5.81; P=0.56) values. Significant differences were observed for yellowness (b*) (females 1.9 vs. males 1.3; P=0.003), Hue (females 0.87 vs. males 0.62; P=0.0001) and cooking loss (females 14.36% vs. males 16.66%; P<0.001) values. These differences could be due to the different slaughter ages of broilers. Results showed that the 100% of the carcasses of the population sampled in this study presented Normal muscles. The association of pH24 and L* as criteria classification can be useful to classify broiler meat quality.

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Meat quality of quail from different genetic groups

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ABSTRACT

To study performance and meat quality of quails, three different trials were carried out: 1) descendents of meat Pharaoh quails selected earlier on the basis of their body weight decrease after periodic deprivation of food (line 1: high decrease of weight, n=10; line 2: low decrease of weight, n=8; line 3: unselected control, n=10); 2) descendents of S18 generation of egg type Japanese quails, selected for low (line 11, n=8) or high (line 12, n=7) yolk cholesterol content and unselected control (line 13, n=11). The decrease of yolk cholesterol content in the line 11 was 313 mg/100 g yolk, and the increase of yolk cholesterol content in the line 12 was 116 mg/100 g yolk, i.e. –17.25% and +6.39% in comparison to parental line 13, respectively; 3) English white quail (B) vs. Manchurian golden quail (M) vs. British range quail (BR). All quail-chicks were grown in a deep litter floor up to 35 days of age, under continuous lighting, with water and feed (commercial diet according to the age) ad libitum until 12h before slaughtering, when feed was withdrawn. At the end of the experiment all birds were weighted and slaughtered. The Pectoralis superficialis (PS) muscle was removed, weighted and stored frozen until to assess cholesterol content and intramuscular collagen (IMC) properties (collagen and crosslink concentrations). For IMC analyses, hydroxyproline and hydroxylysylpyridinoline (HLP) crosslinks (main connective tissue components influencing meat tenderness) were determined. Data were evaluated by the analysis of variance (ANOVA procedure).

Trial 1. Slaughter performance, cholesterol and IMC concentrations were not different between descendents of selected Pharaoh quails (line 1 and 2). Line 2 had higher (P<0.05) HLP concentrations (6.22 μg/mg) and degree of collagen maturation, expressed as HLP crosslink (0.200 mol HLP/mol collagen), than that line 1. Compared to line 1 and 2, Pharaoh control quails had slightly lower (P=0.057) weight (138.17 vs. 146.44 and 140.75 g, respectively), and lower (P<0.05) muscle HLP concentrations (3.72 vs. 4.83 and 6.22 μg/mg) and degree of collagen maturation (0.135 vs. 0.161 and 0.200 mol HLP/mol collagen). No significant differences were noticed in slaughter performance and collagen and cholesterol muscle contents.

Trial 2. Divergent selection for yolk cholesterol content influenced slaughter weight and cholesterol content in PS of Japanese quails, while did not significantly affect collagen properties and PS weight. Compared to line 12, birds of line 11, were by lower cholesterol in the egg yolk, had lower (P<0.05) body weight (105.35 vs. 115.26 g) and cholesterol content (23.57 vs. 37.20 mg/100 g). The control quails (line 13) showed higher (P<0.05) slaughter weight (119.80 g) and collagen content (24.84 μg/mg) that those selected for yolk cholesterol content, while PS weight, cholesterol muscle content, HLP concentrations and the degree of collagen maturation were found to be similar between the three lines. Trial 3. Neither quail slaughter performance nor meat quality was significantly affected by breeds. In conclusion, descendents of meat Pharaoh quails selected on periodic deprivation of food showed that there were clear differences in IMC maturity and muscle HLP concentration; divergent selection for yolk cholesterol content significantly influences growth and the amount of cholesterol in meat. The meat characterized by lower values of IMC maturity and HLP crosslink concentration is thought to be more tender.
Population structure analysis in some Italian chicken breeds

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ABSTRACT

The presented study was aimed to investigate the population structure of four traditional Italian breeds and one commercial hybrid strain, control population, to organise conservation projects and to support protection policies. Blood samples from 199 birds (Valdarnese breed VB=86; Livornese Bianca LB=12; Golden Comet® GC=15; Bianca di Saluzzo BS=50; Bionda Piemontese BP=36) were analysed. Genomic DNA was extracted from blood samples according to standard protocols. All birds were genotyped at 8 microsatellites loci (ADL102, ADL158, ADL176, ADL181, ADL210, ADL267, ADL136, ADL171). Each microsatellite marker was subjected to PCR amplification and PCR products were separated on ABI Prism 377 DNA Sequencer. Allele frequencies and Hardy-Weinberg equilibrium deviation (HW) (P-value) at the eight microsatellite loci were calculated. A model based cluster algorithm was applied using Structure software version 2.2, using a Bayesian algorithm to perform K-means clustering. Based on genotypes microsatellite markers, individuals were clustered into a given number of populations and assigned probabilistically to cluster inferred. The structure software clearly distinguished VB, BS and BP populations based on breed specific microsatellite genotypes. A slightly mixed cluster was calculated for LB population. The GC cluster shows the genetic presence of the different breeds underlining the hybrid origin of these commercial strain. Chicken genetic variability is worldwide endangered, pure breeds conservation aimed to maintain biodiversity and to improve product differentiation is a valuable objective in animal productions. Microsatellites supported by statistic software are powerful tools in genetic diversity investigation: our results show that VB, BS and BP birds are characterized by good genetic variability and clustering ability, at a lower clustering ability level we find LB birds, GC breed showed a mixed origin. Conservation priorities may be successfully planned by using molecular markers investigating populations studies.
Chicken sperm cryopreservation
by the pellet method: study on sperm
working concentration

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ABSTRACT

The purpose of this study was to determine the effects of sperm working concentration during cryopreservation pellet procedure for chicken semen. Semen samples were collected three times a week, by dorso-abdominal massage, from 18 Mericanella della Brianza (local Italian breed) chicken breeders. Two sperm working concentration, 1 or 1.5*10⁹ cells/ml, were studied during semen processing for cryopreservation. Each day of collection ejaculates were pooled in two semen samples, diluted in prefreezing diluent (1 or 1.5*10⁹ cells/ml) and equilibrated at 5°C for 20 minutes; then, dimethylacetamide (DMA; 6% final concentration) was added and equilibrated at 5°C for 1 minute. Following equilibration, the samples were frozen by direct dropping into a liquid nitrogen bath. Frozen semen pellets were transferred into cryovials, stored into liquid nitrogen tank, and thawed in water bath at 60°C. Sperm quality was assessed in fresh semen soon after collection (time 0) and in frozen/thawed semen pellets (time FT). The following sperm quality parameters were measured: subjective motility (%), damaged sperm (%), modified Ethidium bromide procedure using hypotonic solution (“stress test”) and viable sperm (eosin-nigrosin staining). The experimental protocol was repeated in different days of semen collection to increase the number of replicates per treatment (n=4). The data were analyzed by GLM procedure of SAS. Recovery rate of motility (RM), viability (RV) and undamaged (RU) sperm (%) after thawing was calculated. Sperm working concentration did not significantly affect sperm quality after thawing (time FT) and very similar recovery rates in sperm quality were measured processing semen at 1 and 1.5*10⁹ cells/ml (RM=36.30%, RV=39.47%, RU=30.68% and RM=32.33%, RV=35.42%, RU=36.91% respectively). Different works indicated a systematic lack of standardization in cryopreservation procedure. In order to standardize semen processing the initial semen dilution to a fixed working concentration was considered instead of the usual proportional semen dilution. In addition, processing a sperm suspension containing a known cell concentration is considered advantageous if cryopreserved semen will be used for artificial insemination (AI). We suggest to use 1.5*10⁹ cells/ml as sperm working concentration because it allows to store more cells in one semen pellet, therefore thawing step and preparation of the insemination dose for AI will be simplified. Finally, the recovery rate of quality parameters has been used to measure the efficiency of the cryopreservation procedure and it is suggested as reference parameter to compare different studies.

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Identification of the causative mutation of the black non-agouti coat colour phenotype in the domestic rabbit (Oryctolagus cuniculus)

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ABSTRACT

Coat colour genetics has been the matter of a large number of studies that in mice have up to now identified almost 300 loci affecting pigmentation. Among these loci, the Agouti and the Extension loci interact to determine the relative amount of two types of melanin, pheomelanin (yellow-red pigment) or eumelanin (black-brown pigment), in hair and skin. The Agouti locus encodes the agouti signaling protein (ASIP), which is a small paracrine peptide that in wild-type mice is expressed only in specialized cells of the dermis whereas in human and cattle is ubiquitously expressed. In mice as well as in other species, loss-of-function mutations of the Agouti (ASIP) gene cause the production of eumelanin while gain-of-function mutations lead to pheomelanin production. A variety of coat colours appears as a result of these alterations that also show epistatic interactions with MC1R mutations (the Extension locus). In the European rabbit (Oryctolagus cuniculus), classical studies have indicated the presence of three alleles at the Agouti locus: A (wild type allele), a' (black and tan) and a (non-agouti). Here, using genomic DNA obtained from a wild-type coat colour rabbit, we amplified, by long PCR, and sequenced the ASIP gene from exon 2 to exon 4. Then, using DNA isolated from hair roots we resequenced the three coding exons in 13 rabbits of different coat colour (1 Belgian Hare; 2 Burgundy Fawn; 2 Californian; 1 Champagne d'Argent; 1 Checkered Giant with black markings; 2 Giant Grey, 1 Loop with wild-type coat colour; 3 Vienna Blue). One bp insertion, that causes a frameshift of the reading frame in exon 2, was identified in rabbits with black/dark coat colour suggesting that this is the causative mutation of the black non-agouti coat colour phenotype. In order to confirm this hypothesis, we genotyped this mutation in 407 rabbits across 31 different breeds that have been previously analysed for mutations we already identified in the MC1R gene. The insertion in exon 2 was in homozygous state in black animals of a large number of breeds that classical genetic studies indicated to be fixed for the black non-agouti allele. Epistatic interactions between ASIP and MC1R mutations have been observed in two-generation rabbit families.
An interdisciplinary approach to investigate the *English spotting* locus and its association with megacolon in the domestic rabbit: a new putative model of enteric neuronal dysfunction

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**ABSTRACT**

Studies on coat colour genetics in the domestic rabbit, carried out at the beginning of the last century, led to the identification of the *English spotting* locus, characterized by an incomplete dominant mutant allele (En). *Homozygous non mutant* en/en rabbits have solid coloured phenotype. *Heterozygous* En/en rabbits possess far larger patches of coloured fur compared to the homozygous En/En animals. The latter ones may be almost completely white. The En/en genotype is selected for show purposes and a few breeds have a recognized standard, that according to these early studies, should be the result of this allele combination. The En/En genotype seems to be subvital probably due its association with an underlying megacolon syndrome. Here we studied this locus starting from the confirmation of its mode of inheritance and effects on coat colour. To follow the segregation of the *English spotting* alleles, a F1 population was created crossing Checkered Giant rabbits. Chi square test indicated no deviation from the classical Mendelian ratio of 25% (almost completely white animals), 50% (normal spotted animals), 25% (solid coloured animals). However, the extent and position of the patches in the animals classified as “almost completely white” or “normal spotted” varied, probably due to the action of modifier genes. In addition, segments of cecum and colon of two En/En and two en/en rabbits have been harvested. Tissue specimens have been fixed in Zamboni’s solution, embedded in paraffin, cut with a microtome and stained with cresyl violet or prepared as whole mounts and processed for indirect immunofluorescence. These techniques unravelled an apparently normal enteric neural network on both putative megacolon tissues and controls. On the contrary, some other colon specimens of En/En rabbits that were processed for transmission electron microscopy showed important anomalies of enteric neurons and nerve endings. Sequencing of a few candidate genes identified several polymorphisms. In conclusion, these preliminary results suggest a genetically determined enteric neuropathy responsible for megacolon in the investigated rabbit model.
In vitro qualitative characteristics of rabbit spermatozoa after 120 h of solid storage at 5°C and 15°C

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ABSTRACT

Despite the important role that artificial insemination plays in rabbit breeding, this procedure is limited by the short length of time that rabbit sperm can be stored because of the relatively poor fertility achieved both with liquid stored or cryopreserved semen. The use of refrigerated semen is possible just for short periods, normally less than 24-48 h after semen collection, with spermatozoa diluted in liquid extenders and chilled at temperatures between 5 and 25°C. In this last few years investigation on this topic are focusing on the use of solid storage of spermatozoa, provided by gelatine addition to fresh semen extenders. In rabbit, the solid storage seems to prolong sperm preservability, maintaining sperm fertility potential for up 72 h, but little attention has been spent in evaluating storage temperatures effects on quality of solid stored rabbit spermatozoa. For this reason a study was conducted in order to compare the efficacy of 5 and 15°C as holding temperature in lengthen the lifespan of rabbit semen during 120 h of in vitro storage in solid state. Six pooled semen samples ten-fold diluted with a commercial jellified extender were each split into 2 subsamples that were respectively stored at 5°C and 15°C. Total and forward progressive motility (light microscopy), head and tail sperm membrane integrity (SyBr-PI staining and water-test, respectively) and acrosome intact spermatozoa (PSA-FITC staining) were recorded in fresh semen (0 h) and after 48 and 120 h of chilled storage. A different decline in the sperm survival rate and functionality was shown during storage time according to the chilling temperature: at both 48 and 120 h of storage, better values of total (P<0.01) and forward (P<0.05) progressive motility, head and tail membrane integrity (P<0.05) and acrosome intact spermatozoa (P<0.05) were found in semen stored at 5°C compared that one stored at 15°C. Therefore, according to this in vitro study, 5°C were better than 15°C for retain the quality of rabbit spermatozoa held for 120 h in solid state.
Lengthening of the remating interval improves body condition and reproduction efficiency of lactating rabbit does

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ABSTRACT

The requirements of intensively reared rabbit does are very high because of the overlapping of lactation and pregnancy. In this situation, it is important to adopt the most appropriate reproductive system to improve the energy balance of the lactating does and to maximise their productive potential. Previous studies demonstrated that an increase of dietary energy content is often not sufficient to cancel the energy deficit and avoid intense body mobilisation. A negative energy balance is detrimental for the reproductive process and the length of the reproductive life is shorter in intensively than in extensively reared does. Therefore, it seems interesting to study strategies which can reduce the energy mobilisation in lactating females and improve their reproductive performance. This research evaluated body condition, as assessed by some blood metabolites profiles, and pregnancy rate of 120 multiparous hybrid rabbit does submitted to different reproduction rhythms: AI at 11 (I11=60) or 25 (I25=60) d after kindling and weaning at 35 d. Blood samplings (=360) were repeated at 12, 26 and 36 d pp in all the rabbits. Glucose was analysed by the glucose oxidase method (Sigma), non-esterified fatty acids (NEFA) using a two-reaction enzymatic-based colorimetric assay (Wako) and plasma urea nitrogen (PUN) by a colorimetric assay (Sigma). Reproduction efficiency was evaluated by abdominal palpation 15 d after AI to determine the pregnancy rate (PR) (pregnant does x 100/IA). All data were analyzed by GLM SAS-procedure. The trial was repeated for two consecutive reproduction cycles with identical experimental design; however, no significant differences were found between cycles. Throughout the experiment mean concentrations of glucose, the body’s major energy source, and PUN were both lower in I11 compared to I25 (87.6 vs. 115.7 mg/dl; 11.2 vs. 15.8 mg/dl; P<0.05). In contrast, NEFA mean level resulted higher in I11 rather than in I25 (0.313 vs. 0.279 mmol/L; P<0.05). Moreover, NEFA values were consistent with a steadier mobilisation of adipose depots during lactation in I25 than in I11 (0.247-0.283-0.301 vs. 0.283-0.338-0.318 mmol/L; P<0.05). NEFA are released by the action of hormone sensitive lipase on triglycerides stores in adipose tissue and high NEFA concentrations are indicative of negative energy balance. Hence, low circulating NEFA may reflect direction of fat metabolism towards reduced lipolysis due to increased plasma insulin concentrations. The observation that PUN was constantly lower in I11 throughout the trial, suggests that an alteration of protein metabolism occurred during lactation. As a matter of fact, in energy deficient rabbits protein-sparing adaptation comes into play to limit turn-over and degradation of protein, whereas during compensatory higher energy intake, increased protein synthesis is associated with decreased nitrogen excretion and lower plasma urea. PR was higher in I25 does compared to I11 (82.4 vs. 71.3%; P<0.05). PR increased when does were mated later after kindling, suggesting the progressive recovery of reproductive ability during lactation. The present results demonstrate that limiting the nutritional solicitation of females by shortening the length of superposition between lactation and pregnancy, could permit to reduce body stores mobilisation, improving in the meantime body condition and reproduction efficiency.
Evaluation of polymorphism in rabbit FAS and C-KIT genes as a tool for the study of male fertility

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ABSTRACT

Male fertility is greatly influenced by environmental factors. However, large individual variations in reproductive traits are frequently observed between animals of common origin and age kept under the same strictly controlled conditions, as in an experimental group of bucks. Thus, it can be assumed that, even in animal species undergoing genetic selection, genetic diversity, as that due to gene polymorphism, can be at least partly responsible for differences in reproductive efficiency. For this reason, in the present work a candidate gene approach was used to find genetic traits related to individual variability of male rabbit fertility, evaluated by the semen quality. Both studied genes codify for membrane receptors expressed in testis, even if their role in male gonad is not completely clear. FAS molecule is generally known as a death receptor for, upon ligation, may induce apoptosis signalling leading, for instance, to immune-regulatory T lymphocyte death. Recently, however, it was found that FAS can also mediate different non apoptotic functions, involving cell protection and proliferation. C-KIT receptor regulates a variety of biological responses, as cell proliferation, apoptosis and adhesion, leading to important effects on haematopoiesis, melanogenesis and spermatogenesis. According to some authors, murine C-KIT and FAS receptors are expressed by germ cells and their respective ligands, SCF and FASL, by Sertoli cells. It was postulated that, in the testis, FAS/FASL molecules act as a paracrine proapoptotic system, while C-KIT/SCF could be a paracrine prosurvival system. Thus, both systems could be involved in the dynamic balance between cell proliferation and apoptotic cell death that is essential for spermatogenesis, as well as for many other tissues. The present work was aimed to search for polymorphism in FAS and C-KIT genes and to study the presence of these receptors in the mature rabbit spermatozoa, in order to assess the influence of genetic polymorphism on different phenotypic traits related to male fertility. The mRNAs corresponding to complete or partial coding sequences of rabbit FAS and C-KIT genes were retrieved from GenBank database and compared with the homologue genes of human, rat and mouse to deduce exon-intron organization of the considered rabbit gene regions. In order to perform PCR amplification, 6 primer pairs on FAS sequence and 5 primer pairs on C-KIT sequence were designed. To detect polymorphism in the investigated gene regions, DNA of 15 animals was then amplified and sequenced. Up to now, after sequence analysis and alignment, 3 SNPs were identified in C-KIT gene. Ejaculate samples were repeatedly taken from each rabbit in order to assess the sperm cell morphology, concentration, vitality and motility. Furthermore, the vital spermatozoa were immuno-cytochemically stained by anti-FAS and anti-C-KIT antibodies. The staining results were evaluated by computer image analysis, observing different patterns of immune reactivity at single sperm cell level. The associations between FAS and C-KIT genotype and the phenotypic traits concerning the seminal material quality were then investigated.
Meat quality of outdoor reared rabbits: effect of group size

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ABSTRACT

Recently some recommendations were produced from EFSA (European Food and Safety Authority) to respect the health and welfare of rabbit reared for meat production; among these recommendations, the colony rearing is considered one of the most suitable rabbit condition to express the behavioural typical pattern and one of the system in order to increase comfort. The objective of this study was to evaluate, in outdoor colony rearing system, the effect of group size on meat quality of a slow growing local rabbit population. The rabbits were weaned at 35 days and housed with conventional system. At 56±3 days old, 84 rabbits were selected and divided into three groups at random. The groups were transferred outdoor in a wooded area and housed in wire net colony cages. Each colony cages measured 100x150x76h cm. Three different groups size (three replications) were studied: 4 animals/cage (T4), 8 animals/cage (T8), 16 animals/cage (T16) with a stocking density of 5 animals/m². The rabbits were fed a complete feed and alfa-alfa hay ad libitum. At 103±3 days, 12 animals for each group were slaughtered according to WRSA Commission. After 24 hours the loin region and the right hind leg were excised from each carcass and analyzed for assessment meat quality. The pHu was determined in situ on the right Longissimus lumborum muscle at the level of the 5th lumbar vertebra and on the Biceps femoris muscle. Instrumental meat colour expressed as L* (Lightness), a* (redness), b* (yellowness) according to CIELab system was measured with a Minolta CR300 apparatus with a light source D65 on a transversal section of Longissimus lumborum muscle and on the Biceps femoris muscle surface. Water holding capacity was measured on 6 samples of Longissimus lumborum muscle for each group, as cooking loss after cooking in a ventilated oven and in water-bath. The meat quality parameters were analyzed by ANOVA. In T16 group the meat from Longissimus lumborum and Biceps femoris showed significantly higher pHu values than in T8 and T4 groups (5.76 vs. 5.58 and 5.56; 5.85 vs. 5.69 and 5.70, respectively; P<0.01): it might be possible that, as a consequence of a greater locomotory activity during rearing period and during capture for the slaughtering, muscular glycogen decreased and meat acidification was modified. Moreover the lower pHu of T4 and T8 groups could be related to more favourable welfare rearing conditions that reduced the stress. For Longissimus lumborum colour traits and water holding capacity were not significantly affected by group size; for Biceps femoris T16 group showed greater L* values than T8 and T4 groups (55.16 vs. 52.49 and 53.75; P<0.01). In conclusion, considering that the T4 group obtained good performances in a shorter reared period and showed the lack of aggressiveness than the others groups, as reported in previous paper, it is possible to recommend for outdoor rabbit production the lower group size to assure the animal welfare and the good quality of products. Moreover, it is necessary to underline that the higher value of pHu observed in the meat derived from T16 group could modify the product quality during storage and for this reason T4 and T8 group size are more advisable.
Chemical and nutritive characteristics of companion rabbit petfoods available in the Italian market

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ABSTRACT

In Italy rabbits are intensively farmed for meat purpose, whereas in north Europe, USA and Canada they are popular as pet animals, easy to breed in hutches or pens. In recent years, rabbits have become more and more popular as pet animals also in Italy, particularly small breeds such as Dutch, Mini-Lop and Netherland Dwarf. Along with the rise in pet rabbits popularity, increasing diseases have appeared such as obesity, gastrointestinal and dental diseases. These pathologies are often associated with incorrect feeding practices and they represent the most common reason for physical examination at the veterinary practices. Although the domestic market offers several complete foods for dwarf rabbits, with great variability in ingredients, physical form and packaging, animal health disorders are not reducing. The aim of the study was to investigate on the proximate composition, fibre fractions, gross energy, minerals and vitamins D and E contents, fatty acids (FA) profile and aflatoxin content of six complete commercial foods for pet rabbits (A, B, C, D, E, F). Results provided from the analyses were compared and their meeting to the current recommended requirements verified. In terms of crude protein petfoods D (CP=17.2%) and F (CP=18.2%) exceeded the requirements indicated around 12-16%. Petfoods A, B and C were below the recommended level (13%) for crude fibre. The Insoluble Dietary Fibre (IDF) content fitted the requirements in all petfoods. The fat content ranged between 2.3–5%. Starch content exceeded the recommendations (0-13.5%) in food A (28%), B (38%) C (30%) and D (20%). All petfoods reported an adequate minerals supply but the Ca:P ratio in foods E and F exceeded the requirements (2.93 and 2.63, respectively). Vitamin E content was found halved with respect to the general requirements (ranging from 21 mg/kg in food B to 38 mg/kg in food F). No specific FA requirements for rabbits are mentioned in literature apart from a small amount of essential FA, easily met through the amount of lipids contained in the raw materials commonly used for rabbit feeding. The linoleic acid is indicative of the high levels of cereals and oil seed meal whereas linolenic acid result from the lucerne preponderance in the food formulation. Food D and E were low in linoleic acid and high in linolenic acid, resulting in a proper n-6/n-3 ratio (2.3) if compared to food A (13.7), B (23.2) and C (17.8). Aflatoxin contamination was tested in all six petfoods and food C was found positive. This preliminary study has shown a wide chemical variability among companion rabbit’s petfood available in the Italian market indicating a general lack of respect of the rabbit’s nutrient requirements. Further researches are required to provide correct indications to petfood manufacturers.

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Rearing system and performance of wild rabbits in Sicily: preliminary results

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ABSTRACT

In the last years, the rearing of wild rabbit has been considerably intensified, due to an increased interest in wild species and the need to repopulate those areas where the population of wild rabbits has been decimated. The aim of this study was to evaluate the productive and reproductive performance of wild rabbits in Sicily, with the overall objective to optimize the rearing system used by agricultural enterprises. For this purpose, we chose a farm situated in the province of Palermo, comprising a closed structure where both breeding animals and offspring were kept, and an open-air enclosure 25 ha, bounded by a fence in order to prevent predators from entering, and divided into various sections, allowing the rabbits to change section as they liked. Within the enclosure, mangers and drinking troughs were provided in order to decrease as much as possible the mortality due to difficulties of adapting to the external environment. Beyond that, special capturing spaces were created. The young rabbits were kept within the enclosure from the age of 60 days (week 1 after the vaccination for MEV and mixomatosis) until they were reintroduced, or alternatively, they reached the appropriate slaughtering weight of approx. 1.5 kg. The trial was performed from June 2007 to June 2008. From the farm’s total rabbit population (comprising 300 female and approximately 15 male adult rabbits), 60 rabbit does were randomly sampled (20 primiparous, 20 secundiparous, and 20 pluriparous) and housed in standard flat-deck cages within the closed structure; daylight and ventilation were provided for by 4 large lateral windows. In the absence of any artificial illumination, the animals were able to maintain a natural circadian rhythm and their physiological reproductive cycles. The rabbits were subjected to an extensive rhythm, with mating during the post-weaning period of approximately 30 days. Hay and two types of rabbit pellet feed were used, depending on the respective physiological state of the animals, i.e. milking or weaning period. The number of alive pups per litter, the number of pups per parity order, the number of weaned pups per litter, the mortality rate at weaning, and the average weight at weaning were determined. The results suggest that the rearing system used in this study had a positive effect on the rabbits’ reproductive performance. Wild rabbits usually produce litters of 6 pups at most (Fusi, 1993), and the number of pups in this trial resulted 5.4±1.57 (mean ±SD) for the primiparous, 5.33±1.55 for the secundiparous, and 5.28±1.52 for the pluriparous; the number of alive pups per litter resulted 5.31±1.53, and the number of weaned pups per litter was found to be 4.92±1.77. At weaning, the rabbits had an average weight of 386.23 g±64.26; the mortality rate was found to be 7.38%, thus confirming the results found by Alabiso et al. (2006). It is concluded that the rearing system used in this study, in its attempt to respect as much as possible the wild status of the rabbits, showed a satisfying productivity.