

# IMPACTS OF TOXIC SUBSTANCES IN SHEEP AND GOATS IN NORTHERN GREECE

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The increasing demand for products of either in plant or animal origin has led to an intensified agricultural production resulting an increased use of chemical (toxic) substances. These frequently use.... are pesticides and of great concern is their direct or indirect effect on people and animals. In the present study, the influence of toxic substances (pesticides mainly) on sheep and goats is presented, according to region and timeline, i.e. having been brought during the years 1998-2004. For this purpose, 175 biological specimens (stomach content, intestine, liver, kidney, spleen, lung, heart, blood and urine) of 101 sheep, 74 goats suspected of having been poisoned and 83 feedstuff specimens were studied and analyzed in the Animal and Feed Toxicological Laboratory of Veterinary Research Institute of NAGREF. The samples came from the following regions Eastern- Macedonia, Central Macedonia, Western Macedonia and Epirus. The analytical techniques used for the identification and quantification of toxic substances in biological specimens of animals (sheep and goats) and feedstuff, include thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography (GC). Also, atomic absorption spectrometry (AAS) was used for the determination of heavy metals. Sometimes, UV-Vis spectrometry and colorimetry methods were also employed. Data derived from the analysis showed 32.67 % of sheep and 22.97 % of goats proved positive for a toxic substance that caused poisoning to animals. Also 25.3 % of feedstuff tested positive for a toxic substance responsible for animal toxicity. The region with the greatest positive percentage was Eastern Macedonia-Thrace (36.84%) followed by Central Macedonia (25.99%). Finally, the smallest percentage was observed in Western Macedonia and Epirus (16.67%).

It seemed that the group of toxic substances detected in biological specimens of animals (goats and sheep) and feedstuff, with the highest percentage (81.69%), is pesticides. In particular, most of them were carbamates (Carbofuran and methomyl) and organophosphates (methamidophos, terbufos, phorate, methyl parathion), at 52.11% and 22.54%, respectively.

Copper concentrations that were detected in liver samples of sheep were over 500mg/kg (w/w) at 34.72% of examined sheep. These cases of chalcocis observed in sheep, were a result of grazing crops being sprayed with copper products.

In our opinion, the percentage of positive cases in Central Macedonia and Thrace is due to the frequent use of pesticides owing to the great agricultural production in this region. The smaller percentage of positive cases in Western Macedonia and Epirus is due to the less frequent use of pesticides because of the mountainous regions and the resulting limited agricultural activities.

In conclusion, the 27.52% of examined samples of toxic substances were identified as those causing toxicosis. The highest percentage of positive cases was attributed to pesticides (81.69%). The carbamate group was the most common pesticide followed by the organophosphates.

# CASES OF PROVENTRICULAR AND VENTRICULAR IMPACTION IN OSTRICHES

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## Summary

This report describes cases of stomach impaction in ostriches from various regions of Greece. Ostrich breeding started in Greece in 1998 with imported adult ostriches from Italy, Holland, Belgium and Cyprus led to a subsequent local commerce in chicks. Today there are 169 ostrich farms all around the country which have bred 6,445 birds for meat production under intensive conditions.

In the Clinic of Avian Medicine, Veterinary Faculty of Aristotelian Univ. Thessaloniki, 27 dead ostriches were submitted for necropsy, aged from 7 days to 6 years old. In younger ostriches, the clinical signs were loss of appetite, increased water intake, listlessness and death within 1 to 2 days of the onset of the symptoms. In older ones, similar signs of longer duration were initially noticed in addition to dehydration, vomiting, decreased defecation, weight loss, emaciation, sternal recumbency, inability to stand and move, outstretched neck and, finally, death.

The stomach content of 19 ostriches (17 chicks, 1 juvenile and 1 grower) included wooden parts of coarse-cut alfalfa, hard, fibrous roots of the self-growing weed *Cynodon dactylon*, large calamus and hard hays. Also, plastic bands, bags, components of drinking troughs, pieces of artificial grass and nails were found in some of them. The stomach content was ball-shaped, the outside of which consisted of tangled oblong fibrous roots.

In the stomach of 8 ostriches (1 chick, 4 growers and 3 adults) great amounts of sand, gravel and stone were found to have passed into the duodenum. The intestine was empty with a red-dark mucous membrane.

Bacteriological examinations in aerobic and anaerobic conditions were made from the gizzard wall, liver, lung, kidney and yolk sac in blood and McConkey agar. Samples of intestine were cultured into selenite broth and consequently subcultured on McConkey agar plates. Intestinal content was examined parasitologically. No pathogenic bacteria were isolated. Neither parasitic ova nor coccidial oocysts were detected from parasitologic examinations.

Stomach impaction, the most common cause of death in ostriches farmed under intensive conditions, is correlated with ostrich idiosyncrasy and predisposing stress factors such as transport, movement to a new environment, inadequate feed, nutritional deficiencies and imbalances and inadequate management practices. Ingestion of foreign bodies and consumption of long-stemmed grasses are common offenders causing stomach impaction. Impaction cases in adults and juvenile ostriches have been referred worldwide. More rare are the cases involving the 4 chicks aged between 7 and 14 days. What was remarkable was the discovery in their stomach of the fibrous roots of the self-growing weed *Cynodon dactylon*. This weed has very hard and oblong roots and is inappropriate feed for chicks principally and should be removed entirely from the ostrich feeding grounds.

Stomach impaction with sand, gravel and stone is also familiar in adult and juvenile ostriches as the ratites are geophagic, meaning they will pick at and consume any small foreign object

within reach. The nutritional disorientation-deviation is enforced by stress factors such as changes to a different feed or movement to new places and especially in adult birds through an inability to reach a coveted sexual mate.

The impaction from similar content observed in the chicks aged 15 days, rare in intensive conditions, likely resulted from non-recognition of feed, early movement in outside paddock, incorporation into older groups and many other management mistakes due to the inadequate experience of the ostrich owners regarding this new-breeding species in Greece.

Early diagnosis based on palpation and radiography or ultrasonography and appropriate medical treatment can save affected ostriches. Oesophagostomy or gastrotomy could carefully be performed. Stress factors should be avoided and the foreign materials should be carefully removed from birds' paddocks.

# NOVEL MICROSATELLITE SEQUENCES FROM THE SHEEP (*Ovis aries*) GENOME

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During the past decade, highly polymorphic simple sequence repeats (referred to as microsatellites) became the most frequently used molecular markers in genome mapping, parentage analysis, population genetics, conservation of biological resources and forensic DNA studies. They are tandemly repeated DNA sequences of 1-6 nucleotide motifs found dispersed throughout all genomes analyzed so far. Although little is known about their functional role *in vivo*, their abundance, genomic distribution and high allelic variability are well characterized. Aiming to contribute to the pool of potent marker for a detailed genetic mapping of the sheep (*Ovis aries*), a so-called small-insert genomic library has been constructed and screened for microsatellite sequences.

In particular, high molecular genomic DNA has been isolated from leucocytes of a Chios breed ram. Various combinations of Alu I restriction enzyme concentration and incubation time were tested to determine the combination that resulted in a majority of DNA fragments within 1000 bp size. Size fractions of 1000 bp were recovered from a preparative agarose gel using a Qiaquick gel extraction column (Qiagen) and then ligated into linearized pBluescript II KS<sup>+</sup> vector. Plasmid ligations were then transformed into competent DH5a *Escherichia coli* cells and plated on LB-media plates containing suitable concentrations of ampicillin and X-Gal (5-bromo-4-chloro-3-indolyl-bD-galactoside).

The small-insert library which resulted after the wash of grown colonies in LB liquid media was found to possess a titer of  $2 \times 10^7$  colonies per ml and about 1/3 of non recombinant clones. The library was screened using a <sup>32</sup>P- labeled (GT)<sub>20</sub> oligonucleotide probe following standard procedures. Over 30,000 colonies were screened, resulting in 64 potential positive clones containing the (GT)<sub>20</sub> repeat. After a rescreening procedure and *in silico* analysis, 48 clones were found to be positive, containing an average insert of 900 bp. All positive microsatellite sequences were deposited in the GenBank database, revealing 43,000 novel base pairs of ovine genomic sequence which has been determined to harbor 22 potentially polymorphic microsatellite loci. The 68% of the sequenced clones had repeats of sufficient length and flanking sequence to design oligonucleotides and so may be used as primers for polymorphic studies. Two of them, AY743387 and AY743404 were found to have high similarity with the coding region of oncogene DKFZ and DOCK4, respectively.

In conclusion, the construction of the above ovine small-insert genomic library resulted in new informative markers for mapping while the high homology of two ovine microsatellites with genes causing malignancies enables the establishment of new genomes as models for oncogenicity studies.

## EFFECT OF FIELD PEA SEEDS ON BROILERS

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### SUMMARY

A growth experiment was conducted in order to estimate the nutritive value of field pea “Olimpos”. The field pea “Olimpos” is a local variety which grows usually early in spring in northern Greece and has deep green colored flowers and seeds. The variety of “Olimpos” contained 21.0, 1.02, 3.83, 3.81, 0.09 and 0.41% of crude protein, ether extract, crude fibre, ash, calcium and total phosphorus, respectively and 0.70 and 1.69 mg/g of trypsin and hymotrypsin inhibitors, respectively. This experiment was comprised of two periods, the first (1-28 days) and the second (29-42days), in which 180 broilers (90 males and 90 females) were used. During the first period, the broilers were randomly allocated into 3 groups (M<sub>1</sub>, A<sub>1</sub> and B<sub>1</sub>) according to the completely randomized experimental design (C.R). The broilers of group M<sub>1</sub> (control) fed on a typical growing diet based on corn and soybean meal while the broilers of groups A<sub>1</sub> and B<sub>1</sub> fed on diets in which soybean meal was replaced with field pea “Olimpos” at levels of 10 and 20%, respectively. In the second period, the broilers were distributed into three groups (M<sub>2</sub>, A<sub>2</sub>, B<sub>2</sub>). The broilers of group M<sub>2</sub> (control) fed on a typical fattening diet based on corn and soybean meal while the broilers of groups A<sub>2</sub> and B<sub>2</sub> fed on diets in which soybean meal was replaced with field pea “Olimpos” at levels of 20 and 40%, respectively. The intake of food and water was *ad libitum*. No statistically significant differences in growth rate and feed conversion efficiency, nor in carcass yield, bone yield and edible tissue were noticed. The mean daily gain, feed consumption and feed conversion efficiency calculated for all broilers during the entire experimental period were 43.03g/day, 91.7g/day and 2.14kg feed/kg LW, respectively. The mean bone yield and edible tissue of all broilers was 30.9 and 69.1%, respectively. No significant effects on liver condition were found. The results of the present study showed that the field pea “Olimpos” could be used in the feeding of broilers aged 1-28 days at a level of up to 142 g/kg of feed and in the feeding of broilers aged 29-42 days at level of up to 252 g/kg feed, without any adverse effect on either the performance of broilers or the quality carcass traits.

**Key words:** *Broilers, field pea, performance, carcass characteristics.*

# SEASONAL EFFECT ON OOCYTE COMPETENCE AND SEMEN QUALITY ASSESSED USING *IN VITRO* FERTILISATION

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## **AIM**

The objective of the present study was to determine the effect of season of collection on the quality of both gametes (oocyte and sperm) in terms of their ability to produce embryos following fertilization *in vitro*. The experiments were designed to examine the effects of season on oocyte developmental competence and the ability of sperm to fertilize, independently.

## **EXPERIMENTAL DESIGN**

### **Experiment 1.**

The aim of this experiment was to investigate the effect of season on the *in vitro* development of oocytes collected from adult ewes. Semen from 3 rams (Suffolk cross) was collected in March and frozen. A pool of semen from the 3 rams was used in each IVF replicate to avoid specific effects of one animal. Oocytes were recovered from the ovaries of crossbred ewes slaughtered in January, March, May, July, September, November and a minimum of 2 replicates proceed.

### **Experiment 2.**

The aim of this experiment was to determine the effect of season on the fertilizing ability of ram semen *in vitro*. Semen was collected from 4 different rams (Suffolk cross) at 4 times of the year: March (transition into anoestrus), June (anoestrus), September (transition into the breeding season), December (breeding season). For each IVF replicate, semen from 1 ram collected at all seasons was used, to avoid any confounding effects of oocyte batch. A minimum of 3 replicates were carried out for each ram. Oocytes for IVF were collected during August and September.

### **Statistical Analysis**

Data were analysed using the general linear model procedure or the mixed model procedure in SAS (SAS version 8.2, Cary, North Carolina, USA) as appropriate.

## ***RESULTS***

### **Experiment 1**

The only significant effect of season was on blastocyst development at Day 6 ( $P < 0.05$ ). For all other developmental parameters (cleavage rate, blastocyst yield at Day 7 and Day 8) the seasonal effect was not statistically significant ( $P = 0.0882$ ,  $P = 0.2608$ ,  $P = 0.2629$ , respectively).

### **Experiment 2**

Season of semen collection significantly affected oocyte cleavage rate but not the blastocyst yield. The only significant difference in cleavage was between semen collected in March ( $0.52 \pm 0.051$ ) and that collected in June ( $0.65 \pm 0.051$ ,  $P < 0.05$ ).

## ***CONCLUSION***

Despite variations in cleavage and developmental rates of embryos following oocyte or semen collection at different times of the year, there was no significant difference due to season on either oocyte developmental competence or on the fertilising ability of sperm *in vitro*. It is obvious that, despite the apparent seasonal effects on both of gametes *in vivo*, our study failed to demonstrate these differences *in vitro*, suggesting that the dynamics of the system may not allow expression of these differences or that seasonal variations in these parameters were not sufficient pronounced to be detectable.