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BIOAEROSOLS PRODUCED BY WASTEWATER TREATMENT PLANT

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ABSTRACT

Bioaerosols are defined as a collection of aerosolized biological particles which can vary greatly in size, ranging from less than 20 nm to 100 micrometer in diameter. The composition, size, and concentration of the microbial populations comprising the bioaerosol vary with the source, dispersal mechanism in the air, and, most importantly the environmental conditions prevailing at a particular site. The ultimate effect of bioaerosols on human and animal health will depend on the organism characteristics, growth conditions, and organism viability. Air will often contain micro-organisms such as viruses, bacteria, and fungi. Wastewater/sewage treatment, animal farms, farming of land, application of manure, and composting belong among significant sources of bioaerosols.

Key words: bioaerosol; relative humidity; temperature; wastewater treatment

INTRODUCTION

Bioaerosols originating from potentially pathogenic sources may have the potential to cause disease in humans and animals if transported by air currents. Bioaerosols have been linked to highly publicised problems, such as sick building syndrome (SBS) (Hiipakka and Buffington, 3), humidifier fever, farmers lung, tuberculosis, brucellosis, legionnaires disease, Organic Toxic Dust Syndrome and others. Temperature can both increase and decrease bacterial bioaerosol concentrations. Increasing temperature has been found to decrease the survival of bacterial bioaerosols under experimental conditions (Handley and Webster, 2) and in a greenhouse.

Relative humidity is often associated with bacterial bioaerosol survival as well. Increases in relative humidity are known to increase survival (Marthi *et al.*, 5), especially under the influence of direct sunlight (Handley and Webster, 2). Under foggy conditions (100 % humidity) bacterial bioaerosols are thought to propagate in the air, supporting the assumption that a higher humidity increases survival.

MATERIAL AND METHODS

In the experiment, samples were collected by means of a sampler MAS-100 Eco. The MAS-100 Eco air monitoring system is a compact sampler for use with standard Petri dishes. Petri dishes with respective nutrient media are placed on top of the dish support of the sampler and after aspiration of present volume of air, they are incubated at appropriate temperatures. The plate counts were recalculated per 1 m^3 of air.

RESULTS

Samples of bioaerosol were collected from different locations of a municipal wastewater treatment plant in two stages. Simultaneously we measured relative humidity and temperature of the air. In the wastewater treatment plant municipal wastewater is treated by mechanical, chemical and biological processes so it can be discharged into the recipient. Samples collected in

Table I. First stage

Place of samling	Litres sampled	TCB CFU.m^{-3}	TC CFU.m^{-3}	HB CFU.m^{-3}	Moulds CFU.m^{-3}	Yeast CFU.m^{-3}
Influent	5	68.10^2	46.10^2	0	2.10^2	2.10^2
T = 13.3 °C, RH = 82 %	10	26.10^2	23.10^2	0	20.10^2	35.10^2
Coarse treatment	5	76.10^2	12.10^2	0	18.10^2	14.10^2
T = 13.8 °C, RH = 91.3 %	10	7.10^2	29.10^2	0	24.10^2	27.10^2
Fat removal	5	46.10^2	16.10^2	0	40.10^2	78.10^2
T = 12.8 °C, RH = 91.8 %	10	15.10^2	14.10^2	0	23.10^2	30.10^2
Fine mech. treatment (in)	5	32.10^2	8.10^2	0	40.10^2	18.10^2
T = 12.1 °C, RH = 91.8 %	10	28.10^2	13.10^2	0	30.10^2	23.10^2
Fine mech. treatment(out)	5	$292{\cdot}10^2$	132.10^2	0	26.10^2	562.10^2
T = 12.8 °C, RH = 90.3 %	10	$269{\cdot}10^2$	252.10^2	3.10^2	26.10^2	110.10^2
Activation tank	5	6.10^2	0	0	20.10^2	14.10^2
T = 13.1 °C, RH = 84.5 %	10	1.10^2	0	0	17.10^2	6.10^2
Liquid sludge	5	2.10^2	0	0	46.10^2	8.10^2
T = 14.6 °C, RH = 85.5 %	10	2.10^2	0	0	18.10^2	$10{\cdot}10^2$
Dewatered sludge	5	30.10^2	0	0	32.10^2	$14{\cdot}10^2$
T = 15.6 °C, RH = 74.9	10	20.10^2	2.10^2	0	29.10^2	$16{\cdot}10^2$

Table II. Second stage

Place of sampling	Litres sampled	TCB CFU.m^3	TC CFU.m^3	HB CFU.m^3	Moulds CFU.m^3	Yeast CFU.m^3
Influent	5	$24{\cdot}10^2$	$18{\cdot}10^2$	0	0	$10{\cdot}10^2$
T = 11.5 °C, RH = 54 %	10	$19{\cdot}10^2$	$15{\cdot}10^2$	0	$11{\cdot}10^2$	$23{\cdot}10^2$
Coarse treatment	5	$26{\cdot}10^2$	0	0	$8{\cdot}10^2$	$6{\cdot}10^2$
T = 11 °C, RH = 56.9 %	10	$26{\cdot}10^2$	$5{\cdot}10^2$	0	$8{\cdot}10^2$	$7{\cdot}10^2$
Fat removal	5	$24{\cdot}10^2$	$66{\cdot}10^2$	0	$18{\cdot}10^2$	$36{\cdot}10^2$
T = 14 °C, RH = 47.7 %	10	$79{\cdot}10^2$	$11{\cdot}10^2$	0	$19{\cdot}10^2$	$41{\cdot}10^2$
Fine mech. treatment (in)	5	$40{\cdot}10^2$	$2{\cdot}10^2$	$8{\cdot}10^2$	$44{\cdot}10^2$	$108{\cdot}10^2$
T = 13.8 °C, RH = 57.4 %	10	$27{\cdot}10^2$	$14{\cdot}10^2$	$4{\cdot}10^2$	$31{\cdot}10^2$	NC
Fine mech. treatment(out)	5	$22{\cdot}10^2$	$100{\cdot}10^2$	$2{\cdot}10^2$	$32{\cdot}10^2$	$82{\cdot}10^2$
T = 11 °C, RH = 61.7 %	10	$57{\cdot}10^2$	$92{\cdot}10^2$	$5{\cdot}10^2$	$26{\cdot}10^2$	$82{\cdot}10^2$
Activation tank	5	$10{\cdot}10^2$	0	0	$10{\cdot}10^2$	$4{\cdot}10^2$
T = 11.6 °C, RH = 56.7 %	10	$9{\cdot}10^2$	$1{\cdot}10^2$	0	$3{\cdot}10^2$	$8{\cdot}10^2$
Liquid sludge	5	$40{\cdot}10^2$	0	0	$14{\cdot}10^2$	$2{\cdot}10^2$
T = 15.4 °C, RH = 50.5 %	10	$37{\cdot}10^2$	0	0	$15{\cdot}10^2$	$6{\cdot}10^2$
Dewatered sludge	5	$6{\cdot}10^2$	0	0	$6{\cdot}10^2$	$18{\cdot}10^2$
T = 14.6 °C, RH = 49 %	10	$10{\cdot}10^2$	0	0	$8{\cdot}10^2$	$3{\cdot}10^2$

TCB – Total count bacteria; TC – Total coliforms; HB – Haemolytic bacteria; NC – noncountable

the influent location contained increased numbers of micro-organisms associated with the respective contamination of wastewater in this location. In the section of mechanical pre-treatment on rakes and sieves and fine pre-treatment by sand trap, in the enclosed warm and humid environment, where bioaerosol accumulates in a confined space at limited exposure to sunlight that could also devitalise the micro-organisms, there were evidently almost ideal conditions for their survival. The numbers of aerial micro-organisms were lower in the location of aeration tanks and the tank for storage of treated liquid sludge which is most likely related to the previous treatment of waste-water and sludge. Measurements in the outer environment can also be affected essentially by atmospherical conditions. In the building for dewatering of sewage sludge we recorded increased temperature and decreased relative humidity. The latter factors resulted in development of dry bioaerosol.

DISCUSSION

Some areas of wastewater treatment plants produce higher concentrations of bioaerosols than others. The proximity to an aeration source, and its aeration rate have been linked to the level of bioaerosols produced (Brandi *et al.*, 1). Using personal samplers it has been shown that sewage treatment plant employees that have a higher incidence of headache, tiredness and nausea were exposed to more rod-shaped and more total culturable bacteria (Melbostad *et al.*, 6).

The prevalence of self reported gastroenteritis and headache were higher in activated sludge wastewater treatment workers compared to rates for college maintenance and oil refinery workers (Khuder *et al.*, 4). Workers who spent more time in areas where the potential for higher exposure existed (i.e. aeration tanks), had higher rates of illness than those who did not (Khuder *et al.*, 4).

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MONITORING THE DISTRIBUTION AND ABUNDANCE OF *Ixodes ricinus* TICKS IN RELEVANCE OF CLIMATE CHANGE AND PREVALENCE OF *Borrelia burgdorferi* SENSU LATO IN NORTHERN SLOVAKIA (Liptovská valley)

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ABSTRACT

During 2004–2007 a total of 4201 *Ixodes ricinus* ticks were collected by flagging vegetation of a mixed forest in Northern Slovakia (Liptovská valley) to explore the influence of climate change and altitudinal gradients. The first studies (2004, 2007) took place in Northern Slovakia regions from 495 to 1000 m above sea level. The tick population abundance (1.9 to 57.3 ticks per hour, respectively) is comparable to other areas of Western Carpaths. The influence of temperature and humidity on tick density and distribution were investigated in area of Mnich (695–700 above see). A total of 3219 *Ixodes ricinus* were collected at regular two/three week intervals from April 2005 to May 2006. Two peaks of tick activity were recorded in this area the spring peak in May–June (84–128 per hour, respectively) and the autumn peak (21 per hour, respectively) in September. The maximum activity of tick were detected in correlation the air daily temperature 15–20 °C and relative humidity 75–100 mm.

Key words: climate change; daily temperature; *Ixodes ricinus*; relative humidity

INTRODUCTION

Ixodes ricinus is a tick of considerable significance as a vector of both livestock and human diseases. The understanding of the seasonal population dynamics has a major impact on the knowledge of the transmission rates of the many pathogens this tick can transmit (1).

MATERIAL AND METHODS

The study site is an area in north-central Slovakia in the Liptovská valley (495 to 1000 m above sea level). Climate is with mild summers (the average temperature in July 10/16 °C) and cold winters (-5/-9 °C in January). Rains are common (700–800 mm). Tick sampling procedures were performed between 2004 and 2007. The sites was examined for questing ticks by dragging a 100 × 70 cm white flannelette flag over the vegetation for 60 min. Ticks were usually collected at regular two/three week intervals, in the morning (6.00–9.00), at the noon (10.00–15.00) and in the evening (16.00–20.30). The cloth was examined, ticks removed and place in 70 %-ethanol. Recorded densities of larvae, nymphs and adults of *I. ricinus* were used to infer the seasonal and daily dynamics of the tick over the entire period of study. The relative tick abundance was estimated by determining the number of ticks collected per person per 600–800 m^2 (3).

RESULTS

Figure 1 shows the seasonal dynamics of *Ixodes ricinus*. Seasonal numbers of ticks showed a bimodal pattern of activity. The autumn peak (August–September) was

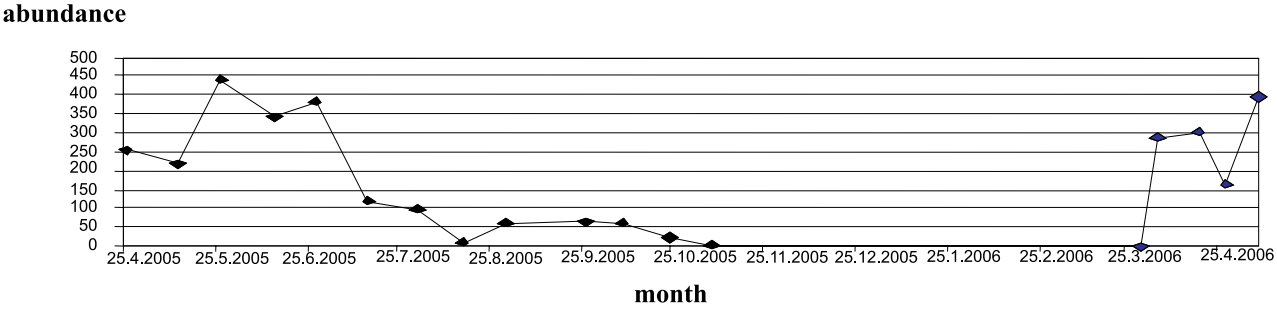

Fig. 1. The monthly evolution of numbers of *Ixodes ricinus* in the site of study between april 2005 and april 2006. Abundance is expressed as the average number of ticks per 600–800 m²

usually smaller than the spring one (May–June, 84–128 per hour, respectively). Two robust correlations between weather data and tick abundance were found, namely peak of activity of *Ixodes ricinus* is correlated with monthly humidity 75–100 mm and daily temperature 15–20 °C. Observation daily acitivity were recorded the significant change during the spring month the April, May, june 2005, where the number of morning dragging of ticks were lower due to temperature.

DISCUSSION

During 2004–2007 were confirmed distribution of *Ixodes ricinus* in Northern Slovakia (Liptovská valley) as firstly ecological report in this region. The tick population abundance (1.9 to 57.3 ticks per hour, respectively) is comparable to other areas of Western Carpaths (3). We observed propagation of tick to the highest altitude. Two peaks of tick activity were recorded in this area the spring peak in May–June (84–128 per hour, respectively) and the autumn peak (21 per hour, respectively) in September, known in other report (4). The maximum activity of tick were detected in correlation the air daily temperature 15–20 °C and relative humidity 75–100 mm, the sesonal distribution is depend of wheather factors (2).

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COPPER ADMINISTRATION ALTERS FINE PARAMETERS OF SPERMATOZOA MOTILITY *in Vitro*

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ABSTRACT

Heavy metals are, in fact, one of the oldest environmental problems, and they are widely distributed in the environmental workplace. Copper is an essential trace element in plant and animal nutrition and copper sulphate ($CuSO_4$) is strong irritant. This study focuses on the effects of this environmental contaminant on fine parameters of spermatozoa motility *in vitro*. Rabbit spermatozoa were cultured with copper ($CuSO_4.5H_2O$) which was added to semen in 5% solution and subsequently diluted 1 : 1–10. Analysis was carried out using a Computer Assisted Semen Analyzer (CASA) system in 2 time periods (0 and 120 minutes). Detailed evaluation of spermatozoa distance (DCL – distance curved line; DAP – distance average path; DSL – distance straight line) and velocity (VCL – velocity curved line; VAP– velocity average path; VSL – velocity straight line) parameters detected significant ($p < 0.05$) decrease in all studied markers in groups with copper addition in comparison with control group at all time periods. Straightness, linearity, wobble amplitude of lateral head displacement and beat cross frequency of spermatozoa were altered weakly. At all time periods (Time 0, Time 60 and Time 120), non-significant decrease of lateral head displacement (in control 2.87–4.75 µm; after copper addition 0–3.37 µm) was detected indirectly confirming progressive motility decrease. At Time 0, Beat Cross Frequency (BCF) significantly ($p < 0.05$) decreased in groups B and I, while after 60 minutes of incubation the decrease was in groups D, F, I and K in comparison with the control group. Findings indicate negative influence of high copper concentrations in semen on fine parameters of spermatozoa motility parameters and thereby supporting the notion for this heavy metal in high concentrations as a risk factor of the environment.

Key words: CASA; copper; motility; rabbit; spermatozoa; toxicity

INTRODUCTION

Chemical environmental pollution is increasing by rapid leaps worldwide due to the development of modern human society. Some metals are essential for life, others have unknown biological functions, either favorable or toxic, and some others have the potential to cause diseases. Heavy metals are, in fact, one of the oldest environmental problems, and they are widely distributed in the environmental workplace (7).

Copper is a reddish metal that occurs naturally in rock, soil, water, sediment, and at low levels, air. Earth's crust comprises of copper (50 ppm on an average) – windblown dust being the primary natural source of emission. Other natural sources of emission include volcanoes, decaying vegetation, forest fires and sea spray (4). Anthropogenic emission sources are nonferrous metal production, wood production, iron and steel production, waste incineration, industrial applications, coal combustion, nonferrous metal mining, oil and gasoline combustion, and phosphate fertilizer manufacture (10).

Human economic activities, involving the production and usage of copper and copper compounds, as well as the consumption of materials (including food) that contain amounts of copper, result in the re-distribution of copper in different environmental media (10). Copper is also found in a range of concentrations in many foods and beverages, including drinking water (1). The usual routes by which humans receive toxic exposure to copper in the form of copper sulphate ($CuSO_4$)

are through skin or eye contact, as well as by inhalation of powders and dusts (26).

Copper sulphate is a naturally occurring inorganic salt and copper is an essential trace element in plant and animal nutrition (12, 13). $CuSO_4$ has a diverse range of usage as fungicide, algaecide, herbicide as well as molluscicide. It can be corrosive to the skin and eyes (3). Long-term effects are more likely in individuals with Wilson&'s disease, a condition that causes excessive absorption and storage of copper (1). Examinations of $CuSO_4$-poisoned animals showed signs of acute toxicity in the spleen, liver and kidneys (3). Injury may also occur to the brain, liver, kidneys and gastrointestinal tract in response to overexposure to this material. When given intravenously, or injected into the vein, as little as 4 $mg.kg^{-1}$ is lethal to rabbits (12). It is also toxic to fish and other aquatic invertebrates, such as crab, shrimp and oysters as well as earthworms in soil (3).

The purpose of the present study was to evaluate the potential influence of copper as a risk factor of environment on fine parameters of spermatozoa motility *in vitro*.

MATERIAL AND METHODS

Rabbit semen was obtained on a regular collection schedule and sample from 5 adult breeding rabbits (SCPV, Nitra, The Slovak Republic) was used. Semen was collected and subsequently diluted (Minitüb, Germany) according to routine methods. After processing, the samples were stored in the laboratory at room temperature (20 °C). Analysis was carried out using a CASA system – SpermVision (Minitüb, Tiefenbach, Germany) equipped with microscope Olympus BX 51, Japan (23).

Copper ($CuSO_4.5H_2O$, cupric sulphate; blue vitriol; blue stone; blue copperas; Slavus Bratislava, The Slovak Republic) was added to semen in 5 % solution (group A) and subsequently diluted 1 : 1–10 (group B – 1 : 1; C – 1 : 2; D – 1 : 3; E – 1 : 4; F – 1 : 5; G – 1 : 6; H – 1 : 7; I – 1 : 8; J – 1 : 9; K – 1 : 10). Each sample was placed into Makler Counting Chamber (depth 10 μm, Sefi-Medical Instruments, Germany) and in each sample the following distance and velocity parameters were evaluated at Time 0, after 60 minutes and after 120 minutes – DCL (distance curved line; μm), DAP (distance average path, μm), DSL (distance straight line, μm), VCL (velocity curved line, $\mu m.s^{-1}$), VAP (velocity average path, $\mu m.s^{-1}$), VSL (velocity straight line, $\mu m.s^{-1}$), LIN (linearity – VSL:VCL), STR (straightness – VSL:VAP), WOB (wobble – VAP:VCL), ALH (amplitude of lateral head displacement, μm) and BCF (beat cross frequency, H_2).

Obtained data were statistically analyzed with the help of PC program Excel and SAS using *t*-test and Scheffe's test.

RESULTS AND DISCUSSION

Detailed evaluation of spermatozoa distance (DAP, DCL, and DSL) and velocity (VAP, VCL and VSL) parameters detected significant ($p < 0.05$) decrease of all studied markers in groups with copper addition in comparison with control group at all time periods (Figs. 1–3). Straightness, linearity, wobble, amplitude of lateral head displacement and beat cross frequency were altered weakly. At all time periods (Time 0, Time 60 and Time 120), non-significant decrease of lateral head displacement (in control 2.87–4.75 μm; after copper addition 0–3.37 μm) was detected indirectly confirming progressive motility decrease. Beat cross frequency significantly ($p < 0.05$) decreased in groups B and I at Time 0 and in groups D, F, I and K at Time 60 in comparison with control group (Figs. 4–6).

Unfavorable effect on the animal health may depend on the kind of element and its dose as well as on the utility orientation. There has been increasing concern about the possible effects of toxic metals on mammalian reproduction (30). The focus of various *in vitro* studies on effects of metals on sperm metabolism and motility have varied from the importance of using culture media free from heavy metals to their possible value as contraceptive agents (7).

In general, the impact of $CuSO_4$ on animal studies largely depends on the route of administration. The embryotoxic or teratogenic effects of $CuSO_4$ appeared to be dependent on the moment of pregnancy when $CuSO_4$ was administered; the effects ranged from degeneration of blastocysts and embryolethality, to middle effects such as *ectopia cordis*, effect on ribs and vertebrae (6). In birds, testicular atrophy increased as they were fed larger amounts of $CuSO_4$. Spermatozoa production was also interrupted to varying degrees (3). Reproduction and fertility was affected in pregnant rats given this material on day 3 of pregnancy (6). Developing embryos were resorbed in pregnant hamsters given copper salts intravenously on the eighth day of gestation. In frogs, higher concentrations of the material caused some behavioral changes, such as secretion of mucous, and discharge of eggs and embryos (25).

Spermatozoa must be motile for their journey to the egg. Sperm motility is a complex phenomenon and its fine regulation by ionic, metabolic and enzymatic factors allows rapid and very specific changes in response to the environment so that fertilization occurs (8). Copper, like many other heavy metals, is toxic to human spermatozoa (15). The potentiation of toxicity of copper seems to be of fairly general occurrence with mammalian spermatozoa (29). Its role in the spermatozoa is unclear, but copper appears to be involved in spermatozoa mobility and it may also act at the pituitary receptors which control the release of LH.

In the seminal fluid, the level of copper appears to fall in cases of azoospermia and to increase in oligo- and asthenozoospermia (15). Copper reduces the oxidative processes and glucose consumption, which reduces or abolishes mobility: this property is exploited in intrauterine devices. The use of copper for male contraception has given rise to experimental implantations at various sites within the male system: lumen of the *vas deferens*, epididymis, seminal vesicle, scrotum and the motility of the spermatozoa was abolished in all cases (24).

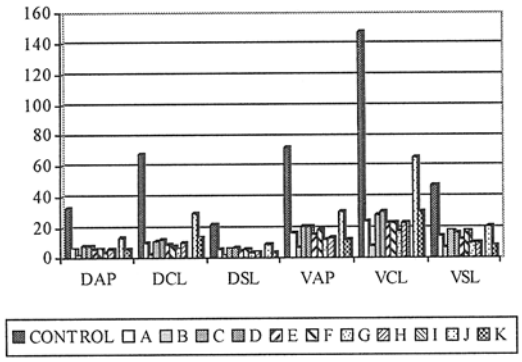

Fig. 1. Distance (μm) and velocity (μm.s^{-1}) parameters at time 0 minutes (distance paraameters expressed in μm; velocity parameters expressed in μm.s^{-1})

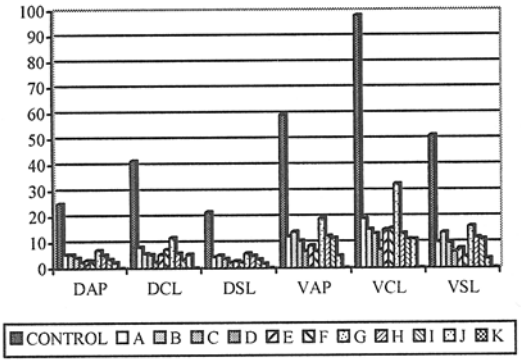

Fig. 2. Distance (μm) and velocity (μm.s^{-1}) parameters at time 60 minutes (distance paraameters expressed in μm; velocity parameters expressed in μm.s^{-1})

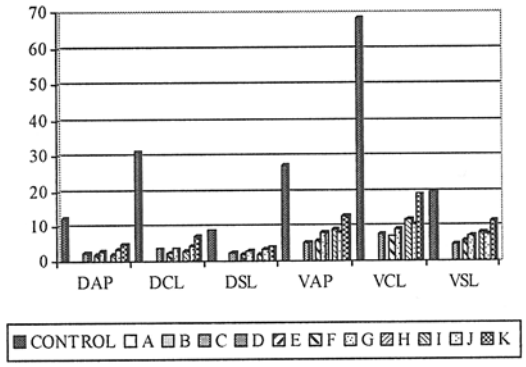

Fig. 3. Distance (μm) and velocity (μm.s^{-1}) parameters at time 120 minutes (distance paraameters expressed in μm; velocity parameters expressed in μm.s^{-1})

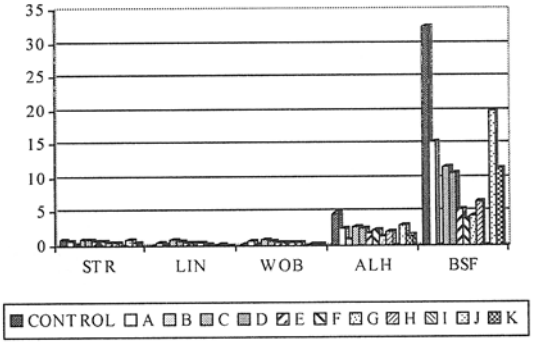

Fig. 4. Straigtness (STR), linearity (LIN), wobble (WOB), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) at time 0 minutes

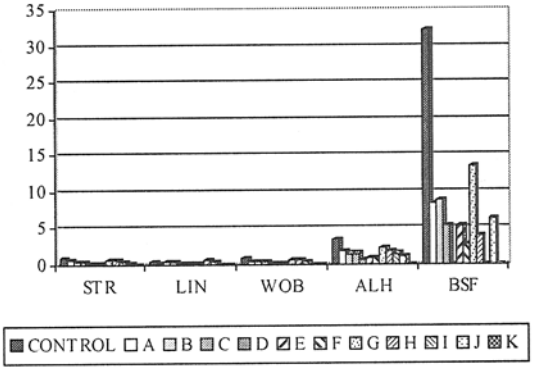

Fig. 5. Straigtness (STR), linearity (LIN), wobble (WOB), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) at time 60 minutes

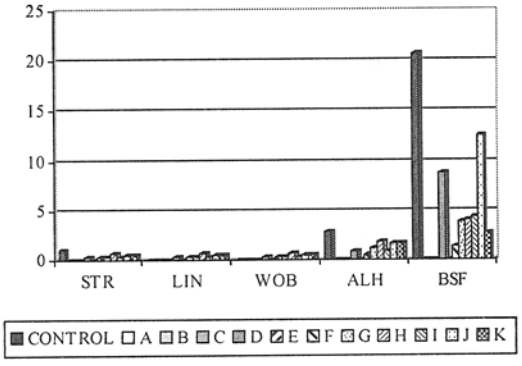

Fig. 4. Straigtness (STR), linearity (LIN), wobble (WOB), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) at time 120 minutes

Morphological abnormality of spermatozoa is often accompanied with weak motility, which is probably attributed more to some factors that co-act on both spermatozoa motility and morphology than to the influence of spermatozoa morphological abnormality on spermatozoa motility (18). Some animals are predisposed to a higher susceptibility to environmental stresses which are known to interfere with normal spermatogenesis (14). Several conditions can interfere with spermatogenesis and reduce spermatozoa quality and production (18). A number of authors have pointed to the role of minerals and heavy metals in spermatozoa. The copper content of the semen also plays a meaningful role.

Traditionally, semen analysis represents one of the

first examinations to be performed for the evaluation of male fertility. Computer Assisted Semen Analyzer (CASA) systems are also superior to other methods regarding the documentation of laboratory values, as all the values are directly obtained online. The accuracy of CASA, as well as Makler chamber manual examination has already been described by some authors (11).

The toxic effects of copper became evident in the destruction of all developmental stages of germinal cells in rams (28). The animals reared near to plants with emission fall-outs containing copper also showed decreased production ability factors (27). The excessive copper intake has a negative effect on the organs of reproduction of males and females (22, 20). The striking power of an organism thus decreases and animals become more susceptible to other diseases.

The effects of metallic copper on human spermatozoa have been described (2). Incubation with the metal caused a fall on the percentage of motile spermatozoa, which was directly related to the surface area of copper employed and to the copper content of whole semen. Low concentrations of ionic copper caused a less marked fall in spermatozoa motility, although the metal was generally more toxic than zinc or cadmium ions (9). A study revealed a statistically positive correlation between copper concentration in semen and progressive motility of spermatozoa (5). In contrast, lower copper concentrations in ejaculates without motility than motile ejaculates were also reported (17).

Several authors stressed the impact of copper on spermatozoa motility (5). A group of researchers pointed out the negative association of copper with semen (16, 19), while others reported a positive correlation between the copper concentration in blood plasma and spermatozoa motility (5).

The degenerative and necrotic effects of copper on the seminiferous epithelium and spermatozoa cells at ram hypercuprosis in industrially exposed areas were confirmed (9). Changes in the ejaculates might indicate the toxic effect to the seminal plasma percentage of motile spermatozoa.

Investigations revealed that the contact of spermatozoa with copper ions probably accounts for their decreased motility (21). In our study, significant decrease of fine parameters of spermatozoa motility in terms of velocity and distance was found in relation to copper addition. This indicates a negative influence of high concentrations of copper as heavy metal on male reproductive functions and thus as a risk factor of environment.

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THE USE OF COMPOSTED VEGETABLE WASTE AS A SOIL MEDIA FOR FREE-RANGE LAYING HENS

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ABSTRACT

A replicated experiment compared the grass ware and bird behavior in two different soil media (90% composted vegetable waste and 10% sand and topsoil) with established grass swards within the range area of a free-range laying hen unit. The rate of loss of grass from the plots was significantly greater within the compost-based soil media plots than the topsoil plots ($P < 0.05$). When birds were first introduced to the fenced areas they showed all behaviour elements with more frequently typical signs of calmness and comfort within the compost plots ($P < 0.05$). Once the birds had prolonged access to the unfenced different swards birds showed a clear preference to the compost plots ($P < 0.05$). It was concluded that composted vegetable waste could have a practical use as a soil media for free-range hens in accordance with on farm welfare and sustainability principles.

Key words: composted; free-range; laying hens; vegetable waste

INTRODUCTION

In developing countries, the word "waste" is often an inappropriate term for organic matter, which is often put to further use. The economies of most developing countries dictate that materials and resources must be used to their full potential, and this has propagated a culture of recycling. Food industry produces a large amount of vegetable waste. Currently they have to sell it to recycling companies in turn recycle the waste and re-sell it on the commercial market for a profit. It is therefore relatively easy to find a free-source of vegetable waste. Vegetable waste compost can be used directly, or with some modification, as a soil medium and good grass swards can be established (5, 6). This gives the small-scale farmer a great opportunity to decrease the expenses with saving the environment. On the other hand, there is a requirement that free-range laying hens must have continuous day-time access to open-air runs that are mainly covered with vegetation (1). Therefore, a good quality sward is important in that it provides some forage for the birds at range and it avoids the pasture becoming muddy. There is a need to examine whether the use of composted vegetable waste has the potential to be used as a soil medium in the range areas and whether its use is practicable without posing any welfare problems for the birds. The principal aim of this study was to establish whether composted vegetable waste has potential for use as a soil medium for free-range commercial units in accordance with the welfare principles existing for free-range laying hens.

MATERIAL AND METHODS

The study was conducted at the Animal Research Institute of the National Agricultural Research Foundation (N. AG. RE. F.), in Giannitsa, Greece. The poultry unit was comprised of a mobile, environmentally-controlled laying hen house that covered all relevant requirements of EU Directives (1). Vegetable waste was provided by a Greek Food Industry. Backyard composting at the farm level was used as a simple technique that requires only suitable organic waste, space to construct the heap and time to carry out the necessary work. The waste was placed in a pit (2 m × 2 m × 1 m deep) and left to decompose for 3 months.

The experimental pasture was subdivided into six plots each of 4 m^2 and 20 cm of the topsoil was removed and replaced with one of the two soil media : a) a mixture of 90 % recycled composted vegetable waste and 10 % sand covered three of the plots and b) topsoil obtained from the same site covered the other three plots. The relative positions of each of the soil media plots within the main pasture area were chosen using random numbers. The same grass seed mixture was used to seed each of the two different soil media. No fertiliser or chemical applications were made to the grass during the experimental period. Grass score was described as the proportion of grass vegetation that covered each plot area.

Observational data of the laying hens were collected from the established plots during using the general procedures outlined by Martin and Bateson (2). The first series of observations was made to measure hens' behaviour specific elements and the second one was made to determine the relative preference of the hens for the swards grown on the two soil media.

Inferential statistics (Pearson's Chi-Square, Mann-Whitney U nonparametric test and independent samples *t*-test) were used to compare treatment differences (4) and all statements of significance are based on a probability of less than 0.05. Statistical analysis was performed using SPSS (3) software.

RESULTS AND DISCUSSION

Grass swards established successfully in each of the two soil media and there was complete grass cover on each of the plots of the experimental pasture when the birds were given access them. There were no visual differences in the condition or growth of the swards between the two soil media. However, the soil below the sward of the compost-based soil media did appear to be somewhat darker in colour. The grass sward gradually decreased over the one month course of the experimental period. The rate of loss of grass from the plots was significantly greater ($P < 0.05$) within the three compost-based soil media plots than the topsoil plots.

No statistical differences have been proved for most of the behavioral elements of the birds between the soil and the compost plots ($P > 0.05$). However, hens had statistically different foraging behavior observed in higher frequency in topsoil plots than in compost ones ($P < 0.05$). On the contrary, dust bathing and standing comfort behavior observations were of higher frequency in the compost plots ($P < 0.05$). The birds used in this experiment had had a long experience of ranging on grass grown on topsoil and evidently had an initial aversion to the recycled vegetable waste swards.

Once the birds had prolonged access to the different swards (unfenced areas) there was a significant difference in the number of birds that frequented the different sward types ($P < 0.05$). Birds indicated that their initial preferences were soon overcome and showed a clear preference to the compost plots when introduced to the six plots of the unfenced experimental pasture.

In conclusion, this study has given preliminary evidence that composted vegetable waste can be transformed into value added soil media for free-range hens. The outlined concept can be naturally transferred to several areas of livestock production.

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EFFECT OF DIETARY ESSENTIAL OIL EXTRACT ON BLOOD VARIABLES OF BROILER CHICKENS

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ABSTRACT

A study was conducted to investigate the effect of different doses of *Cinnamonum zeylanicum* in diet on blood chemistry of broiler chickens. The chicks received basal diets for chicks supplemented with 0%, 0.1%, 0.05% and 0.025% cinnamon for 38 days. Addition of cinnamon to the diet caused a significant lower plasma glucose level and dietary intake of 0.05 and 0.025% cinnamon reduced serum ALT and plasma potassium levels.

Key words: blood variables; broiler chicken; cinnamon

INTRODUCTION

Many herbs and plant extracts have antimicrobial activities (3) and antioxidant properties (6) which make them useful as natural additives in animal feeds. Essential oils derived from sage, rosemary, thyme and other herbs inhibit osteoclast activity and *in vitro* leading to an increase in bone density (4). Recently, it was reported that some essential oils such, as cinnamon has the potential to favorably influence the insulin system and has beneficial effects on blood glucose and lipid metabolism of people with type 2 diabetes (2). There is a dearth of literature regarding the effects of cinnamon on blood biochemistry of broiler chickens.

The objectives of the current study were, therefore, to investigate the effects of different doses of *Cinnamonum zeylanicum* in diet on blood biochemistry of broiler chickens.

MATERIAL AND METHODS

Thirty two 1-d old male broiler chicks were randomly distributed into groups of 8 birds in each one. The chicks received the diets from the day of hatch to 38-d of age. The four types of diets included basal diets for chicks supplemented with 0 %. 0.1 %. 0.05 % and 0.025 % cinnamon (Cinnamoni aetheroleum of *Cinnamonum zeylanicum*, Calendula a.s., Nová Ľubovňa, Slovakia), certificate of quality No. 610. Biochemical parameters were determined by spectrophotometric methods. Results are presented as mean ± S.E.M. Statistical significance was performed by a one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Dietary addition of cinnamon (0.1, 0.05 and 0.025 %) to broiler chickens for 38 days resulted in a decreased plasma glucose level in a dose-dependent manner.

It was found that cinnamaldehyde in *C. zeylanicum* was effective in decreasing plasma glucose concentration and increased plasma insulin to streptozotocin-induced male diabetic wistar rats (7).

Effect of *C. zeylanicum* on blood glucose and insulin sensitivity were evaluated in several animal studies (1). On the other hand, Sambaiah and Srinivasa (1991) (5) reported that common spices did not show any cholesterol lowering effect when added in the diet of rat at about 5-fold the normal human intake.

Table 1. Effect of dietary intake of *Cinnamonum zeylanicum* oil on biochemical parameters in broiler chickens

Item	Control group	0.1% cinnamon	0.05% cinnamon	0.025% cinnamon
Calcium (mmol.l^{-1})	1.53 ± 0.11	1.80 ± 0.17	1.70 ± 0.14	1.60 ± 0.12
Potassium (mmol.l^{-1})	6.74 ± 0.37	6.55 ± 0.66^{cd}	4.12 ± 0.34^{abc}	6.04 ± 0.30^{ad}
ALT (μkat.l^{-1})	1.53 ± 0.09^{ab}	1.25 ± 0.01	0.88 ± 0.21^{a}	0.95 ± 0.09^{b}
Glucose (mmol.l^{-1})	14.80 ± 0.55^{ab}	8.79 ± 0.35^{acd}	11.79 ± 0.63^{bc}	12.45 ± 0.74^{d}
Triglycerides (mmoml.l^{-1})	0.55 ± 0.036	0.51 ± 0.01	0.52 ± 0.03	0.48 ± 0.03

Significant differences within a row are indicated by the same superscript letter
$P < 0.05$; mean ± S.E.M., n = 8

Cinnamon has been investigated in human studies for its antidiabetic properties (2, 9). On the other hand, Vanschoonbeek *et al.* (2006) (8) did not observe a significant change in blood sugar or lipid profile markers. The discrepancy between our data and results of other animal experiments could be due to a number of factors, including differences in effect of cinnamon between species, cinnamon concentration, cinnamon source (quality and chemical characterization), animal genetics or sex.

This prelimitary study about effects of different doses of *Cinnamonum zeylanicum* on blood biochemistry showed that 38 d administration of cinnamon caused a significantly lower plasma glucose level by broiler chickens.

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AGGRESSIVE BEHAVIOUR OF DOGS AND ITS ETHOLOGICAL FUNCTION

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ABSTRACT

Aggressive behaviour is a typical feature of dogs and is important in nature for survival of individuals, their integration into pack, defence of territory, food or the young. Aggression and the related manifestations in humans and animals is an important philosophical, psychosocial, ethological and ethical issue on both human and subhuman level.

Key words: aggressive behaviour; dog; ethology

INTRODUCTION

From the ethological point of view, aggressive behaviour of dogs can be characterised as an intensive, emotional, expressive response oriented against the object of dog's frustration. Aggression is a phylogenetic, pre-domestication, instinctive state of animals of self-preservation importance (4). Dogs are carnivorous animals and as such they exhibit various forms of aggressive behaviour.

PRESENTATION OF THE PROBLEM

According to whether the aggressive behaviour is directed against conspecifics or against individuals of other species, we divide aggression to *intraspecific* and *interspecific*.

Intraspecific aggression comprises ritualised (suppressed) and non-suppressed – obvious aggression oriented against individuals of the same species. It establishes social hierarchy and division of territories of respective animal species in certain area.

Interspecific aggression **is oriented against different animal species and includes offensive and defensive elements. Its most frequent form is the self-protective aggression.**

On the basis of studies of a number of authors (3, 6, 5) the aggressive behaviour of dogs was divided to 8 most frequently occurring forms. These eight types of dog aggression are related to *fear, dominance, possessive behaviour, defending behaviour* (defence of territory, humans), *predatory behaviour, punishment, pain and intraspecies aggression.* Such multitude of categories points to various neurobiological mechanisms of dog aggression.

Defensive aggression

Defensive aggression occurs when the dog defends itself or perceives the situation as threat or pain. Defensive aggression is related to fear, pain or threat of punishment and includes defensive or submissive signals before or during the aggression.

Dominant aggression

Dominant aggression is related to social relationships between two or more animals one of which appropriates important resources, such as food, shelter, mate, and similar. Relationships are established in the group of animals and are maintained on the basis of threat and communication signals which decreases the fighting resulting in injury.

Possessive aggression

This aggression develops when the dog appropriates some objects, for example a bone, food, a toy. Such behaviour is frequently associated with dominant aggression.

Protective aggression

Aggression of this type occurs as a result of territorial behaviour regarding humans or animals.

Intraspecies aggression

Typical intraspecies aggression includes aggression within the group of males and group of females. Less frequently it involves aggression between a male and female.

Predatory aggression

Predatory aggression is a part of feeding behaviour of carnivores. However, some dogs exhibit behaviour which is part of predatory behaviour, e.g. visual exploration, hunting small animals or even humans. The stimuli that usually induce this behaviour include small size and/or movement.

Fear and phobia

Fear affects a wide spectrum of causes (1). Stimuli that cause fear may be related to predators, intensive physical environmental stimuli and warning signals. Fear and phobias can sometimes be observed due to constitutional factors or temperament, or in other cases because of insufficient experience with fear stimuli.

Table 1. Percentage of Specific Aggressive Behaviours Occurring in Each Type of Aggressive Behaviour Problem (2)

	N	Bark	Growl	Bare teeth	Snap	Bite	Stare
Fear	86	60.5	53.5	10.5	24.4	33.7	0.0
Dominance	73	27.4	84.9	23.3	30.1	60.3	20.6
Possessive	65	7.7	69.2	12.3	20.0	33.9	0.0
Protective	63	61.9	52.4	3.2	19.1	25.4	1.6

In addition to the above mentioned forms of normal aggressive behaviour of dogs there are also aggressive ethopaties. They are conditional on predisposing factors (inherent, heritable, developing during perinatal period), immediate factors, such as traumas, infections of CNS, and can also be related to various frustrations and distress.

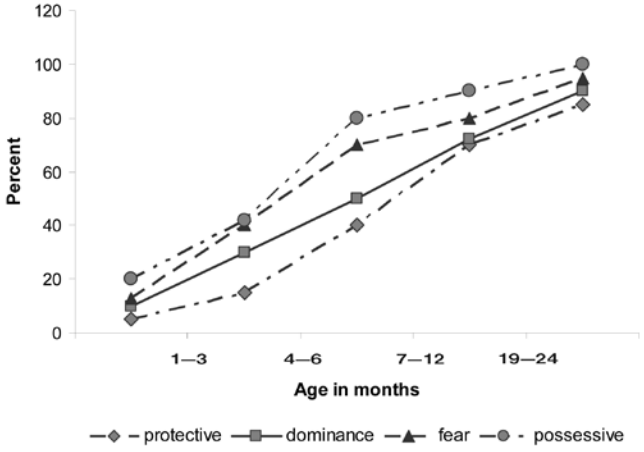

Fig. 1. Percentage of dog exhibiting types of aggressive behaviour problems at different ages (2)

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OROSTHENIC SYNDROME IN ADULT CATTLE: A MINIREVIEW

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ABSTRACT

Stereotypic oral manifestations are characterised by various motoric activities of the tongue. Etiology of these manifestations is based on monotony of environmental stimuli and predisposition in ruminating animals. The most frequent forms include tongue playing, homosexuality of digestive type and excessive licking.

Key words: cattle; minireview; *orosthenic syndrome*

INTRODUCTION

The orosthenic syndrome belongs to a very wide category of cattle ethopaties. These stereotypic oral manifestations are characterised by various motoric activities of the tongue. Their aetiology is based on monotony of environmental stimuli and predisposition of ruminants to this syndrome.

Stereotypies are defined as relatively invariant, apparently functionless, repetitive behaviour (14). They occur as a response to strong stimuli (6) and serve to alleviate its effect (2). It was observed that stereotypic behaviour decreases the physiological response to invoking factors (18). The frequency of occurrence of stereotypies decreases with increasing variability of the environment (14).

Tongue playing

Evaluation performed on 17 farms in Slovakia showed that tongue playing was the most widely spread behaviour disorder in cattle. Milk sucking rated as second (11). Tongue playing likely develops due to suppression of such important oral activities as sucking, grazing and ruminating. It has been assumed that nutrition deficiencies also play an important role (5). The occurrence of orosthenic syndrome increases in tethered animals (8). Similar conclusions were presented also by other authors which considered deficiency of structural fibre in feed and limited movement due to stanchion housing to be the principal factors inducing such behaviour (10). Experimental results proved that restriction of fibre associated with decreased time of food intake contributes to development and frequency of oral stereotypies in cattle (17).

Homosexuality of the digestive type

In common practice this ethopaty is known as intersucking of milk ("suckers") or self-sucking in adult cows. It is classified as a sucking reflex stereotypy with umbilical and inguinal area of one animal serving as a substitution key stimuli for another animal (7). It is an important problem in cattle rearing as it can result in damage to the udder, mastitis decreased milk yield and culling of animals (13).

Evaluation of 18 herds with various types of housing in Slovakia showed that the occurrence of suckers ranged between 0.55 % and 8.33 % (3). Higher number of suckers was observed on farms where calves feed from buckets (9), in dairy cows which showed intersucking in the heifer-age (13), and in animals housed at excessive density (9). The causes of intersucking are multifactorial and include both external factors (way of feeding during milk nutrition, ratio of concentrate and bulk feed, feeding, housing) and internal factors (breed, genotype, life experience).

The sucking itself can be divided to active sucking, when the animal looks for and sucks its partner; reciprocal sucking

when two animals suck each other and passive sucking when one animal allows other to suck itself. There is also chain sucking involving several cows at the same time, tandem chain sucking or sometimes as many as three cows sucking the fourth one (two from sides, one from behind). Self-sucking can also be observed sporadically (cow standing with a lifted limb, or lying). During sucking a foam is produced which is well visible in a stall. Excrements of active sucking cows have similar colour and consistency as those of calves. However, the frequency of urination, defaecation, drinking, length of lying and rumination correspond to average values in the herd (1).

Excessive licking

Psychogenic dermatoses that are well-known in dogs and cats (17), animals in zoos and humans (4) are rare in ruminants. Two forms were observed in cattle (19). One of them is excessive licking of teats and udder, leading to ulceration and even necrosis of teats and udder skin between the teats. It seems that excessive licking of teats and udder in dairy cows has also genetic component (18). This abnormal behaviour can arise due to stress factors (calving, weaning, inadequate socialization or environmental factors).

The second, less frequent form of self-destruction behaviour is licking of shoulders (19).

CONCLUSION

Abnormal behaviour is considered an indicator of worsened welfare. Despite housing under identical conditions there are always individuals which do not respond by stereotypies. The reason for this is obviously a different perceiving of environmental factors by individuals based not only on previous experience but also on genetic predisposition. Therefore absence of abnormal behaviour is not a proof of "good" welfare. The orosthenic syndrome is a diverse and at the same time frequent group of forms of abnormal oral behaviour of cattle and therefore it is important to study its aetiology, prevention and potential control.

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MICROBIOLOGICAL CONTROL OF DISINFECTION EFFICIENCY IN FISH PROCESSING FACILITIES

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ABSTRACT

Disinfectant Topax 66 (it is produced by Ecolab, s.r.o., Bratislava) was tested under laboratory conditions and in fish processing facilities. Under laboratory conditions, *B. cereus* was devitalised after disinfection with 0.1 % solution and 20 min exposure time. When using the same concentration of the disinfectant, *E. coli* and *S. aureus* were devitalised after 5 min exposure time. Good disinfectant effect was recorded in the fish industry, however, surfaces of ancillary equipment were positive for *E. coli*. Sanitation of the facilities was reflected positively in aerial microbial findings as the samples taken were negative for *E. coli*.

Key words: disinfection; efficiency control; Topax 66

INTRODUCTION

The cleaning and disinfectant preparations used in the food industry should be able to devitalize target micro-organisms already at very low concentrations, should not leave residues nor put additional load on the environment. Evaluation of the level of hygiene and sanitation in food industry facilities must be related to individual sectors and production/processing procedures taking into consideration their specificity and ensuring correct interpretation of results. The most objective parameter is determination of microbial contamination which allows one to evaluate the effectiveness of the disinfectant used.

MATERIAL AND METHODS

We tested a disinfectant Topax 66, which is a liquid, alkaline disinfectant containing active chlorine, intended for application with foam production in the food industry in concentration of 2% of the active ingredient. The following Standard Collection microbial strains were used for testing: *E. coli* (CCH 5172); *S. aureus* (CCM 2012).

Suspension test

First we prepared 100 ml volumes of 5 different dilutions of the disinfectant Topax 66 (2.0 %, 1.0 %, 0.5 %, 0.1 %, 0.01 %). Using a pipette, we transferred 0.1 ml of the respective bacterial culture into each of 5 test tubes and added to them 9.9 ml of the respective dilutions. After 5, 20 and 60 minutes, we transferred a loopfull of the respective suspensions into individual test tubes containing 5 ml of nutrient broth. This way inoculated broth was incubated in a thermostat for 24 h at 37 °C.

Microbiological control of effectiveness

Microbiological swabs were taken in production facilities from 10 cm^2 area into sterile saline using sterile cotton swabs. Subsequently, in a laboratory, we inoculated the respective solutions onto meat peptone agar (TPC – total plate counts), Endo agar (*E. coli*) and Sabouraud agar (molds). After 24 h incubation in a thermostat at 37 °C we determined total plate counts and *E. coli*. Determination of moulds was carried out after 5-day incubation at 22 °C.

Table 1. Testing of bactericidal effectiveness of Topax 66 on some bacterial strains by a suspension test

Preparation	Exposure (min)	Concentration (%)				
		0.01	0.1	0.5	1.0	2.0
E. coli	5	+	–	–	–	–
	20	+	–	–	–	–
	60	+	–	–	–	–
S. aureus	5	+	–	–	–	–
	20	+	–	–	–	–
	60	+	–	–	–	–

Table 2. Mean plate counts of micro-organisms detected in the frozen fish section before and after application of Topax 66 (CFU.10 cm^{-2})

Place of sampling	Before disinfection			After disinfection		
	TPC Ø	*E. coli* Ø	Moulds Ø	TPC Ø	*E. coli* Ø	Moulds Ø
Wall	2	112	59	0	0	0
Ceiling	10	0	5	0	0	0
Floor	286	187	100	6	0	1

RESULTS AND DISCUSSION

Cleaning of facilities and technological equipment in food production is an essential part of overall hygiene and has a direct effect on the results of disinfection. Also residues of the processed, hygienically unobjectionable materials, participate to a considerable degree on contamination of food processing premises. They produce a layer on the surface of equipment which is referred to as biofilm. The surface biofilm is a nutrition-rich layer that supports multiplication of micro-organisms in the respective environment (1, 2, 3, 4).

To ensure sanitation of the investigated object we used preparation Topax 66 heated to 40 °C. Results of suspension test, carried out under laboratory conditions, showed bactericidal effect of Topax 66 on *E. coli* and *S. aureus* at 0.1 % concentration and 5-minute exposure (Tab. 1).

Tab. 2 shows results of microbiological swabs taken from surfaces of technological equipment from the frozen fish section. The highest number of colonies was found for TPC which ranged from 2 to 286 CFU from the area of 10 cm^2. After disinfection the total plate counts were in the range of 0–6 CFU and also the plate counts of *E. coli* and moulds were lower.

The importance of microbiological control of disinfection effectiveness is documented in Table 3 which shows reduction of the number of micro-organisms on the surface of ancillary equipment after cleaning and disinfection.

Table 3. Mean plate counts of micro-organisms detected in swabs taken from ancillary equipment in the section of fish salads before and after disinfection with Topax 66 (CFU.10 cm^{-2})

Place of swabbing	Before disinfection			After disinfection		
	TPC	*E. coli*	Moulds	TPC	*E. coli*	Moulds
Push cart 1	NC	11	12	55	0	1
Push cart 2	NC	7	5	0	10	0
Push cart 3	NC	0	22	75	0	17

After operation the numbers of micro-organisms were again considerably increased.

CONCLUSION

Testing of disinfectants under laboratory and food operation conditions is justified because the use of unsuitable preparations or incorrect concentration may result in poor devitalisation effects.

Laboratory testing of the disinfectant Topax 66, intended for disinfection in food production, showed that it was capable of devitalising the tested bacterial strains *E. coli* and *S. aureus* already at 0.1 % concentration and 5 min exposure. This concentration devitalised *B. cereus* after 20 min. exposure. Good disinfectant effects were observed also after disinfection in fish processing facility but presence of *E. coli* was detected on the surface of the ancillary equipment.

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STABILIZATION OF SEWAGE SLUDGE USING ALCALINE AND ACIDIC CHEMICAL PREPARATIONS

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ABSTRACT

A study was conducted to investigate the effect of chemical substances on improvement of hygiene properties of sewage sludge. The chemicals used included H_3PO_4 and lime which belong among substances with disinfectant effect. These substances increase hygiene level of sludge and improve its properties important for application to soil by increasing its agronomic value in terms of important nutrients.

Key words: agricultural soil; lime; phosphoric acid; sewage sludge

INTRODUCTION

Sewage sludge is organic waste containing valuable nutrients (nitrogen, phosphorus and other substances) which justifies their use for application on agricultural soil. However, it also contains components that pose risk to soil, water and food chain when present in increased concentrations (pathogens and toxic elements – heavy metals). With regard to the above mentioned the sewage sludge should be used with caution to prevent contamination of soil, increase crop yields and prevent accumulation of risk substances in the soil and their excessive deposition to landfills with all potential negative consequences (2, 4). The Act No. 188/2003 of the Civil Code on application of sewage sludge and bottom sediments restricts the use of sludge particularly with regard to its content of heavy metals and pathogens.

MATERIAL AND METHODS

In the experiments with stabilisation of sewage sludge we used powder lime with commercial name KOVAP (K+VK) and waste dust retained on electrostatic separator during lime production, marked KOVAS J (K+V) and H_3PO_4 (K+P), all in 3 % concentration, and unamended sludge served as a control (K). All the substrates were stored at laboratory temperature (16.–19.3 °C).

Bacteriological examination of influent and effluent from wastewater treatment plant included determination of plate counts of mesophilic, coliform and faecal coliform bacteria (STN 83 0531-4 and STN-ISO 9308-2) on solid cultivation media (Endo agar, Imuna, Slovakia) and faecal streptococci in sludge (STN-EN ISO 7899-2) on Slanetz-Bartley agar (Biomark, India).

RESULTS AND DISCUSSION

Two hours after addition of lime we recorded increase in pH to 11.3 (K+VK) and 11.1 (K+V) in comparison with control sludge (5.9) and sludge with phosphoric acid which showed decrease to pH 4.6. The different course of pH affected also chemical and microbiological parameters.

The amendment of sludge with lime affected the plate counts of coliform bacteria (KB) which were not found in sludge as soon as two hours after the addition of lime to the respective substrate K+VK. After four hours we were not able to recover coliform bacteria neither from

K + VK nor from K + V. Similar development was observed also with faecal coliforms.

On day 20 these bacteria were present in lowest number in the control sludge ($6.6.10^5$ CFU.ml^{-1}). On this day the bacterial plate counts in the remaining substrates were higher by two orders of magnitude in comparison with the control and even higher than at the beginning of the experiment. This can be ascribed to the important influence of high pH in the first half of the experiment.

The most important action of both types of lime was observed with faecal streptococci as they could not be recovered 2 hours after addition of lime neither from K + VK nor from K + V. The differences were significant ($P < 0.01$) compared to control sludge and sludge amended with phosphoric acid. In the latter sludge the pH rapidly declined to 4 and faecal streptococci decreased by one order within 24 hours.

In agreement with Mignotte (3) our results confirmed that devitalisation effect of lime on sludge depended first of all on pH and the period of its persistence and less on the percentage of added lime. This aspect is important especially when using less valuable materials when the quantity added should be sufficient to reach the appropriate pH (1).

The results obtained showed that addition of waste dust from production of lime KOVAS J (V – 3 % by weight) had no significant devitalisation effect on the observed indicator micro-organisms. We observed reduction in their plate counts 48 h after its addition and within the following 24 hours the micro-organisms started to revitalise again and their numbers began to grow.

CONCLUSION

The powder lime with commercial name KOVAP (VK) showed a partial devitalisation effect when added to sewage sludge. Coliform bacteria were not recovered 2 hours after its addition, but evidently by day 6 of storage they began to grow again when the pH of the respective substrate started to decrease.

The addition of phosphoric acid had no significant devitalisation effect despite decrease of pH in the initial stages of the experiment. The use of H_3PO_4 does not seem to be prospective also with regard to the fact that the sludge itself already contains higher concentration of phosphorus which prevents it application to agricultural soil year after year.

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REDUCTION OF THERMAL LOAD IN PIG FATTENING HOUSE WITH TUNNEL VENTILATION

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ABSTRACT

The effect of tunnel ventilation on reduction of thermal load in stable for fattening pigs with combined air inlet in hot summer period was evaluate on the basis of measuring of microclimatic parameters. The stable had 2 sections with 6 pens with total capacity 500 housed pigs. Basic microclimatic parameters were noticed in each section in 1st, 3rd, 4th and 6th pen in animal zone by ALMEMO 2290-4 device. In fattening house was registered lower average temperature (36.6 °C) than external air temperature (37.6 °C) during operating all fans. Average internal air relative humidity 28.6% was higher than external air humidity by 4.5%. Average internal velocity of air presented 1.22 m.s^{-1} in animal zone. Cooling effect for pigs was ensured by elevated air flow at presented temperature.

Key words: fattening pigs; microclimatic parameters; thermal load; tunnel ventilation

INTRODUCTION

Pigs are relatively sensitive to high environmental temperatures because they cannot sweat and are relatively poor at panting (5). A lot of research has been done on the factors affecting heat production in pigs (3). Reduction in the associated thermal effect of feeding is an efficient mechanism to reduce heat load (11). Generally it is recommended to raise pigs at temperature 3 °C higher than lower critical temperature (10). It was found out that pigs modify their posture in relation to ambient conditions (6). Evaporative heat loss might occur either *via* respiratory evaporation or by evaporation from the wet body surface of pigs (8). Fattening pigs preferred to lie on slatted floor at high ambient temperature (1). Optimum ranges of microclimatic parameters for finishing pigs in Slovakia are presented in the regulation No. 230/1998 (9). Air temperature as critical environmental factor is influenced by relative humidity and air flow velocity. Air humidity level is very important in cooling process (7). In stables for fattening pigs with cross ventilation there have been noticed higher temperatures and lower relative humidity as optimum range in summer period (4, 2).

Possible method of cooling during high ambient temperatures is utilization of enhanced air flow. The cooling effect of air movement is typically expressed by effective temperature, the temperature that animals actually feel (12).

MATERIAL AND METHODS

The aim of this work was to evaluate effect of tunnel ventilation on reduction of thermal load in stable for fattening pigs with combined air inlet for summer and winter period. This system of ventilation was not realized in Slovak conditions up to now.

The stable was longitudinal divided into 2 sections with total capacity 500 housed pigs in 6 pens from 30 to 100 kg. Air exchange in each section in the summer period was provided by 2 front and 2 side fans and by 1 fan for underslatted exhaust situated at backside of stable. External air input was from scope controllable openings situated in front wall. Incoming outlets by their area nearly covered whole internal

vertical front wall intersection and their immediate largeness was adaptable by means of folded curtain.

Reduction of thermal load was evaluated on the basis of measurement of microclimatic parameters in hot summer period. Temperature, relative humidity and air velocity were measured in each section in 1st, 3rd, 4th and 6th pen (K1, K3, K4 and K6) in animal zone 500 mm above floor level by ALMEMO 2290-4 device. During measurement all fans were operated. External air parameters were registered too.

RESULTS AND DISCUSSION

At average external temperature 37.6 °C average temperature in animal zone was 36.6 °C. Average internal temperatures in evaluated pens were lower by 0.5–1.7 °C than the average external temperature. The highest average temperature was noticed in K3 pens (37.1 °C) and the lowest in K6 pens (35.9 °C). Relative humidity of interior air was lower than the low limit of optimum (50 %) by 25–18.5 %. It was as consequence of low humidity of soaked external air (24.1 %) and because humidification was not used in the stable. The highest relative air humidity was noticed in K6 pens (31.5 %) and the lowest in K3 pens (25.7 °C). Average air velocity in animal zone was 1.22 $m.s^{-1}$ at the range of 0.20 to 2.14 $m.s^{-1}$ (in K6 and K4 pens). The highest average value did not exceed upper limit (2.0 $m.s^{-1}$) according to the regulation No. 230/1998 (9).

In our previous evaluations of microclimatic parameters we noticed higher interior air temperatures than external ones in all stables for fattening pigs with cross forced ventilation, although temperatures of external air did not exceed 30 °C in summer period (2). It was proved true fact that the process of evaporative cooling during our measurement was effective (7), therefore in evaluated stable with tunnel ventilation housed pigs tolerated better higher temperatures at lower relative humidity of interior air.

CONCLUSION

In evaluated farrowing house with tunnel ventilation (without humidification) we noticed lower average internal temperature by 1.0 °C than external temperature (37.6 °C) during extreme hot weather. Enhanced air flow velocity in animal zone ensured evaporative cooling of housed pigs. Pigs tolerated better higher temperatures under interoperation with low relative humidity of air (28.6 %). It favourably influenced the reduction of thermal load.

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ECOLOGY AND DOMESTICATION

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ABSTRACT

The present paper focuses on issues related to the effort to describe domestication by means of various categories of relationships defined by ecologists. We prefer the term mutualism, but for more complex understanding in relation to domestication, we consider it suitable to supplement it with a cultural component. On the basis of this, although included among mutualistic interactions, such description of domestication differs from the "common" understanding of mutualism.

Key words: animals; domestication; man; mutualism

INTRODUCTION

Domestication is an interesting phenomenon studied by various scientific branches (9). First domestication dates back approx. to the year 13 000 BC (7). Gradually a potential "accumulated" which has affected the subsequent stages of human development. This potential can be presented, for example, in the form of material reserves or locomotory energy (3). Owing to this the man can resist better to adverse environmental fluctuations.

PRESENTATION OF THE PROBLEM

In ecological books, e.g. (1), (5), domestication is categorised by signs +/+ and by the term mutualism. Odum and Barret (5:366) presented an interesting thought: *"Domestication leads to a special form of mutualism"*. An important element was the word "special" which, as it will be shown later, plays an important role in the context of mutualism-domestication. In the papers dealing with domestication in the biological context we find two terms that are frequently interchanged – the previously mentioned mutualism and symbiosis (4, 6). Boucher *et al.* (2:315) already in the introduction to his paper differentiated the two terms: *"the term mutualism is defined as an interaction between species that is beneficial to both of them"* and *"symbiosis is the living together of two organisms in close association"*.

Already in these definitions we can see some difference between these two terms in the intensity of the relationship, namely between the man and another organism. West *et al.* (8) concentrated on the semantic aspect of the term and their conclusions can be summarised as follows: *"Mutualisms is a special category of cooperation where the signs +/+ are used for a specific case of interspecies cooperation"*.

On all accounts, we will prefer the use of term *mutualism* in the above presented form for description of interaction man/animal in the context of *domestication*. Begon *et al.* (1) compared interaction between the man and domesticated animals/plants to the similar relationship between ants and mushrooms (human agriculture), ants and butterflies of the family *Lycaenidae* (cattle rearing). This raises a question – is domestication of animals and plants by humans really similar to relationships involving ants?

Melinda Zeder (9) offered an interesting view of domestication: *"Domestication is a form of biological mutualism which was transformed from highly developed capacity of the man to the effect of behavioural change across learning and cultural transfer"*.

Owing to this the author clearly perceives domestication as a specific form of biological mutualism, but exceeds its definition in that that the human side includes, in the majority of cases, greater component of intentionality than it is assumed in mutualism. The text clearly shows that the relationship is asymmetric with man playing more important role. And this is exactly the element which we wanted to stress by using the word "specific".

CONCLUSION

The aim of the paper was to point to and to stress some problems related to description of *domestication* when looking through the ecology prism. We focused namely on the interspecies relations and for description of *domestication* we preferred the term mutualism. Subsequently we stressed the necessity to differentiate this specific form of *mutualism* from its "common" form by accentuation of its specific cultural component.

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THE EFFECT OF LONG-TERM AEROBIC STORAGE OF DOGS' EXCREMENTS WITH OR WITHOUT DUST REJECTS FROM LIME PRODUCTION ON THE SURVIVAL OF MODEL HELMINTH EGGS

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SUMMARY

The effect of long-term storage (73 days) of dogs excrements mixed with hay in the ratio of 1 : 5 with or without dust rejects from lime production on the survival of model helminth eggs was studied under laboratory conditions. An application of dust rejects to the mixed dogs' excrements at a concentration of 20 g.kg^{-1} of organic wastes, resulted in a devitalisation of 65.65 ± 2.84 % and at a concentration of 70 g.kg^{-1} devitalisation of 77.05 ± 2.36 % of model unembryonated *Ascaris suum* eggs within 24 hours. *A. suum* eggs were totally devitalised as early as within 8 days in dogs' excrements after application of dust rejects at a concentration of 70 g.kg^{-1} and within 21 days after application of dust rejects at a concentration of 20 g.kg^{-1}. Devitalisation of 87.23 ± 3.21 % of eggs was obtained in the control without dust reject in the end of experiment. For the sanitation of organic wastes from animal production and dogs' excrements, the use of dust rejects from lime production, at more affordable price than quality lime dust, is very suitable.

Key words: *Ascaris suum* eggs; dogs excrements; rejects from lime production; sanitation

INTRODUCTION

Regarding the spread of helminthoses, domestic animals (dogs, cats) are of great importance because they live in a close contact with man. Through faeces of infected dogs and cats the germs of zoonotic helminths spread into the environment. Infection and way of transmission of the disease depends on the way of breeding and on the breeding environment where the animal occurs. On the other hand, animal excrements can supply other essential plant nutrients and serve as a soil amendment by adding organic matter. The most serious problem is the sanitation of this organic wastes.

The aim of our study was to study the effect of long-term aerobic storage of dogs excrements with or without dust rejects from lime production on the survival of model helminth eggs under laboratory conditions.

MATERIALS AND METHODS

Dogs excrements mixed with hay in the ratio of 1 : 5 and mixed with dust rejects from lime production caught on the electrostatic precipitator in a concentration of 20 g.kg^{-1} (D20) and 70 g.kg^{-1} (D70) were used in the experiment. The "artificial contamination of organic wastes" with unembryonated *A. suum* eggs was used approach to make sure that there was a sufficient number of positive samples in our observations. Eggs were inoculated at a dose of 1 000 eggs per carrier into polyurethane carriers, prepared according to Plachý and Juriš (3) and introduced into the wastes. The physical and chemical analysis of organic wastes was performed according to the standards for industrial composts (4). The C content was calculated according to the content of organic matter (OM) by the method of Navarro *et al.* (1), and the C : N ratio was calculated.

Table 1. Physical and chemical properties of the mixed dogs' excrements without dust rejects from lime production

Exposure (Days)	pH	DM (%)	NH_4^+ ($mg.kg^{-1}$ DM)	N_t ($mg.kg^{-1}$ DM)	C : N
0	9.08 ± 0.01	35.66 ± 1.83	219.07 ± 55.70	40758.43 ± 1416.02	11.15:1
1	8.57 ± 0.01	34.66 ± 0.11	232.05 ± 23.57	39116.17 ± 207.87	11.24:1
2	9.61 ± 0.01	35.20 ± 4.23	395.72 ± 2.48	41116.07 ± 1205.26	10.12:1
3	9.78 ± 0.01	37.56 ± 1.93	309.78 ± 95.04	44207.73 ± 3222.05	9.05:1
7	9.01 ± 0.01	37.17 ± 0.29	370.89 ± 8.22	23346.91 ± 5147.86	18.06:1
8	9.39 ± 0.02	33.21± 0.18	82.25 ± 2.55	12152.63 ± 77.34	32.74:1
9	9.55 ± 0.02	29.81± 3.03	132.58 ± 72.91	6556.92 ± 818.39	54.92:1
10	9.49 ± 0.03	31.96 ± 1.13	124.07 ± 0.16	6217.49 ± 27.34	57.31:1
14	9.34 ± 0.03	53.90 ± 4.33	138.03 ± 7.41	5435.17 ± 2904.64	76.40:1
73	8.51 ± 0.03	86.31 ± 0.23	28.09 ± 3.26	9159.80 ± 1327.01	46.33:1

Table 2. Physical and chemical properties of the mixed dogs' excrements with dust rejects from lime production in a concentration of 70 $g.kg^{-1}$

Exposure (Days)	pH	DM (%)	NH_4^+ ($mg.kg^{-1}$ DM)	N_t ($mg.kg^{-1}$ DM)	C : N
0	8.41 ± 0.05	37.21± 0.01	400.57 ± 47.84	45177.35 ± 4724.10	9.64:1
1	11.21 ± 0.02	44.47 ± 0.91	12.51 ± 8.65	32262.4 ± 4212.96	9.66:1
2	9.34 ± 0.03	56.45 ± 15.15	36.17 ± 7.84	16218.95 ± 2664.67	12.44:1
3	8.58 ± 0.01	57.28 ± 33.23	645.10 ± 362.56	45266.01± 19058.77	4.23:1
7	9.08 ± 0.01	45.11 ± 6.79	225.45 ± 91.01	22104.48 ± 11603.9	15.37:1
8	9.13 ± 0.01	43.90 ± 2.84	439.78 ± 141.28	18254.61± 1760.78	16.74:1
9	9.27 ± 0.05	68.66 ± 1.32	398.59 ± 2.51	18083.74 ± 303.53	20.86:1
10	9.12 ± 0.04	64.15 ± 0.16	349.02 ± 10.08	16966.79 ± 191.73	17.70:1
14	8.91 ± 0.03	60.02 ± 0.98	338.50 ± 24.95	17963.19 ± 457.92	14.71:1
73	8.69 ± 0.01	89.06 ± 0.01	74.54 ± 26.60	13972.75 ± 1214.80	22.56:1

Table 3. Physical and chemical properties of the mixed dogs' excrements with dust rejects from lime production in a concentration of 70 $g.kg^{-1}$

Exposure (Days)	pH	DM (%)	NH_4^+ ($mg.kg^{-1}$ DM)	N_t ($mg.kg^{-1}$ DM)	C : N
0	9.08 ± 0.01	35.66 ±.83	219.07 ± 55.70	40758.43 ± 1416.02	11.15:1
1	12.58 ± 0.04	43.11 ± 1.51	41.04 ± 42.79	13041.67 ± 2498.12	16.22:1
2	12.68 ± 0.01	46.81 ± 0.17	10.19	4555.21 ± 936.46	42.69:1
3	12,64 ± 0.01	44.10 ± 1.95	20.65 ± 14.40	6868.28 ± 1649.01	32.67:1
7	12,36 ± 0.01	45.50 ± 0.21	140.34 ± 9.77	26830.72 ± 6801.00	7.73:1
8	9.63 ± 0.01	45.22 ± 0.48	131.32 ± 2.98	14216.80 ± 5981.61	15.04:1
9	9.12 ± 0.01	45.60 ± 1.23	82.59	16161.23 ± 10202.44	16.18:1
10	9.06 ± 0.02	46.75 ± 1.09	85.65 ± 10.62	13399.86 ± 759.04	14.14:1
14	8.82 ± 0.01	52.21 ± 1.08	14.28	19371.98 ± 1147.30	9.21:1
73	8.97 ± 0.02	87.17 ± 0.47	2.20 ± 0.08	12440.27 ± 566.93	24.43:1

Table 4. Damage of *A. suum* eggs during the long-term storage of dogs excrements

Exposure (Days)	Damaged *A. suum* eggs ($\bar{x}$ % ± SD)		
	Control	P20	P70
0	12.62 ± 1.14	12.62 ± 1.14	12.62 ± 1.14
1	35.70 ± 2.46	65.65 ± 2.84*	77.05 ± 2.36*
2	54.43 ± 10.66	68.65 ± 3.89	82.30 ± 4.81
3	67.00 ± 2.55	75.15 ± 1.21	87.60 ± 3.98
7	72.65 ± 4.03	76.25 ± 5.41	97.13 ± 3.97
8	79.80 ± 2.71	76.93 ± 2.69	100
9	81.87 ± 2.90	82.30 ± 4.81	100
10	82.85 ± 4.03	85.69 ± 1.45	100
14	81.96 ± 3.26	95.69 ± 6.35	100
73	87.23 ± 3.21	100	100

$\bar{x}$ – mean values, SD – standard deviation, * – significance at the level $P < 0.01$

Samples were collected after 0, 1, 2, 3, 7, 8, 9, 10, 14 and 73 days of the storage. Three samples were taken and analysed at the given sampling intervals.

RESULTS AND DISCUSION

The following changes in physical and chemical properties of the organic wastes during the long-term aerobic storage were monitored: the pH, dry matter (DM), ammonium ions (NH_4^+), total nitrogen (N_t) and C:N ratio. The physical and chemical properties of the treated material are given in Tables 1–3.

Our experiment showed that stabilisation of organic wastes with dust rejects result in complete devitalisation of *A. suum* eggs (Table 4). The most important physico-chemical factors affecting viability of helminth eggs include pH and ammonia. We observed the highest pH and ammonia content especially in the organic wastes treated with dust rejects. Pescon and Nelson (2) also reported that environmentally relevant concentrations of ammonia may significantly increase the rate of *Ascaris* eggs inactivation during alkaline stabilization.

The issues of safe sanitation and waste management are highly topical as it has been universally acknowledged that the majority of endoparasitic germs is able to cause infection in animals and humans even a year or two later.

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QUANTITATIVE DETERMINATION OF RODENTICIDE RESIDUES BY HPCL METHOD

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ABSTRACT

The importance of determination of rodenticide substances results from their toxic properties. The aim of the present study was to examine quantitatively the soil and cereals for residues of brodifacoum which was applied in the form of commercial preparation BRODER produced by DeArt s.r.o., SR, at the beginning of the vegetation period.

Key words: brodifacoum; residues; rodenticides

INTRODUCTION

An essential principle of protection of the environment is prevention of entry of extraneous substances into its individual components. The development of pesticides is oriented in such a way so their action is directed only on the respective rodentic target species. Considering this aspect it is possible to state that this requirement has not been fulfilled when developing the currently used rodenticidal preparations (6). All currently used rodenticides are toxic to humans and farm animals. Rodenticides are chemicals which enter the environment and food chain due to deliberate human activities (4). With regard to environmental protection and anticipated undesirable effects of chemization, the factors limiting the use of rodenticides include in particular the toxicological risk arising from their residues, development of resistance to rodenticides in the target pests and factors affecting their application (2).

Considerable attention has been paid to observation of residues in the environment and food (1, 8). According to the onset of toxic effects, the currently used rodenticides are divided to acute and chronic. The range of currently available acute rodenticides is quite narrow due to their high toxicity. New generation rodenticides are mostly acting as anticoagulants. After their uptake the animal dies because of haemorrhage into body cavities which is not supposed to be painful and simulates natural death. One of such rodenticidal preparations is brodifacoum (3, 5, 7).

MATERIAL AND METHODS

Anticoagulant brodifacoum, a hydroxycoumarin, is an active ingredient in a preparation recommended for rat control. BRODER G is considered an efficient rodenticide effective also against warfarin-resistant rats, *Rattus norvegicus*. The commercial preparation comes in the form of red coloured, impregnated granules from grain meal which contain 0.005 % brodifacoum. It is used in agriculture and for the control of urban rodents. The bait contains low concentration of rodenticide and is ready for quick use. The preparation was applied to the soil at the beginning of March when sowing the grain (wheat) at a dose of 100 g.m^2 and 500 g.m^2 which, when recalculated per the content of active ingredient in the bait, amounted to 0.5 g.m^2 and 2.5 g/m^2 brodifacoum in the soil, resp. The preparation was applied to soil placed in wooden boxes (1 × 1 × 0.5 m) and controls were prepared in the same way.

During the vegetation period no agrotechnical interventions were made. At the beginning of August, in the harvest period, we collected samples of soil and of grain (entire spikelets). Samples of untreated soil served as a control.

Tab. 1. Residues of brodifacoum in the examined samples of wheat

Sample No. and year of sampling	Applied dose and active ingredient	Crop	Residuum (ppm)
1 2004	Broder G 100 g.m^2 brodifacoum	Wheat	0.018
2 **2005**	Broder G 100 g.m^2 brodifacoum	Wheat	0.012
3 **2006**	Broder G 100 g.m^2 brodifacoum	Wheat	0.022
4 **2004**	Broder G 500 g.m^2 brodifacoum	Wheat	0.044
5 **2005**	Broder G 500 g.m^2 brodifacoum	Wheat	0.034
6 **2006**	Broder G 500 g.m^2 brodifacoum	Wheat	0.043
7 **2004**	Control no aplicate	Wheat	–
8 **2005**	Control no aplicate	Wheat	–
9 **2006**	Control no aplicate	Wheat	–

Determination of brodifacoum residues

Observation of pesticide residues in the environment and food is a subject of considerable attention (1, 8). The most frequently used method for determination of brodifacoum is high performance liquid chromatography (HPLC):

10 g samples were collected, homogenized and extracted for 15 min in 25 ml of 1 : 1 mixture of chloroform and acetone. The solvent was decanted through a filter paper. The extraction was carried out twice and the combined extract was evaporated in a rotating vacuum evaporator at 40 °C. The residuum after evaporation was dissolved in 1 ml of mobile phase to remove the co-extracted proteins. The solution obtained was transferred to a centrifugation tube containing n-hexane. The content of tubes was centrifuged twice for 10 min at 2000 r.p.m. The bottom layer was filtered and used for HPLC analysis.

The chromatographic analysis was carried out on column LiChrospher® 100 RP-18 (5 µm) with acetonitrile (A) and acetate buffer, pH 4.6 (B) (50 + 50) as a mobile phase for warfarin at a flow of 1 ml.min^{-1}, injecting 10 µl aliquots. UV detection was carried out at 310 nm for warfarin and storm and at 265 nm for bromadiolon and brodifacoum.

RESULTS AND DISCUSSION

Samples of soil and above-ground part of wheat were collected at harvest in the stage of harvest ripeness, i. e. at the beginning of August. The total above ground mass of cereals at individual applications served as one sample. Wheat samples No. 1 from 2004, No. 2 from 2005 and No. 3 from 2006 originated from the crop that was obtained after application of rodenticide at a dose of 100 g.m^{-2} Samples No. 4 from 2004, No. 5 from 2005 and No. 6 from 2006 were collected from the crop that was obtained after application of rodenticide at a dose of 500 g.m^{-2} and the control samples No. 7 from 2005, No. 8 from 2006 and No. 9 from 2007 were collected from the cereal crop grown on soil without application of rodenticide (Tab. 1).

Occurence of brodifacoum residues in wheat (ppm)

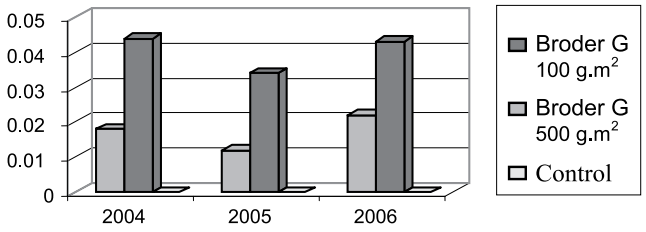

Fig 1. Rodenticide residues and the corresponding dose of BRODER applied to the soil

For each exposure we sowed in parallel control grain without application of rodenticides which was sampled and used as a control. In agreement with the applied dose of rodenticides, we detected the lowest level of brodifacoum residues in samples No. 1, 2 and 3, namely 0.012–0.022 ppm, at a dose of 100 g.m^{-2} of rodenticide preparation. After application of 500 g.m^{-2} of rodenticide to soil the residues in wheat samples No. 4, 5, 6 ranged between 0.034 and 0.044 ppm (Fig. 1).

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VETERINARY ASPECTS IN CONSERVATION MANAGEMENT OF THE ARABIAN ORYX *(Oryx leucoryx)*

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ABSTRACT

In 1998 a new semi-free ranging population of Arabian oryx (*Oryx leucoryx*) was established on the premises of Al Maha Desert Resort (AMDR). A total of 72 animals were introduced into a 25 km^2 fenced desert ecosystem. Animals were donated from different private and governmental establishments. The aim of the project was to establish a healthy population of the Arabian oryx living in an ideal environment and to create a nucleus of animals for future reintroductions. The population reached 200 individuals in 2003 and the resort area was expanded by an additional 120 km^2. The population is managed with minimum human interference. The AMDR, named after the Arabic translation of the name of the Arabian oryx, also serves as an eco-tourist site and aims to promote Arabian wildlife and to encourage environmental protection. The author describes the stages of the field work, as well as difficulties related to management and veterinary care during the reintroduction process.

Key words: *Arabian oryx;* reintroduction; population; veterinary management

INTRODUCTION

After great conservation efforts the world population of Arabian oryx has become stabilized at the beginning of the new millennium and serves as a good example of how to save a species that almost became extinct. The United Arab Emirates (UAE) probably holds the largest part of the world population of Arabian oryx. Thanks to a suitable desert environment and environmental awareness programs, the idea of establishing a free living Arabian oryx population exists at all levels of society in the country. The first idea was to manage surplus animals from different existing and expanding collections and to create a genetically diverse population living with minimum human interference. Because most of the collections are located near to premises with domestic animals, the risk of transmissible disease threatened existing populations. Cases of brucellosis and tuberculosis have been transferred from domestic animals to captive herds of Arabian oryx (1, 7).

The environment of Al Maha Desert Resort

The area was predominantly used for grazing camels in the past and had relatively abundant natural vegetation represented by akacia "salam" tree (*Acacis tortilis*), ghaf tree (*Prosopis cineraria*) and Sidr tree (*Ziziphis spina-chirsti*) providing shade for the introduced oryx. Large sand dunes as well as hard ground covered with gravel comprise 2/3 of the area. A sufficient amount of easily accessible underground water provides suitable irrigation sources for additionally planted indigenous trees. Twenty five square kilometres were fenced by galvanized wire game fences. Four animal grids were placed on each side of the resort. Six feeding spots and two water pools were built around the places with abundant vegetation to provide food and water for the animals.

Introduction of the Arabian oryx

Seventy two animals selected from different privately owned UAE collections and imported from overseas zoological gardens represented the nucleus population. Donating collections were known to be free from infectious disease. All animals were ac-

climatized for desert conditions and had been in the country for more than 6 months before arriving at AMDR. Oryx arrived in winter months in mixed groups of 15–25 animals and were released into holding enclosures.

Socialization, adaptation to the new food and environment were the aims of the 1 month stay in the holding enclosures. To increase body condition alfalfa pellets and hay with mineral supplements were provided before the release from holding pens. Quarantine and health screening protocols were introduced (9, 2). A serology survey for brucellosis and tuberculosis was conducted prior to release and all animals were negative. All animals were dewormed and vaccinated with a polyvalent clostridium vaccine on the day of arrival. One of the main objectives of the project was to leave and observe the animal's behaviour with as little human interference. Wounded or diseases animals were removed from the population. All dead animals were collected and examined post-mortem.

In the first stages the largest problem was to prevent animals from escaping by maintaining the integrity of the fence. During strong desert storms the sand was blown from under the fence pylons and large gaps occurred. From the veterinary point of view heavy infestation by ticks was the first problem that was noticed, mainly in sub-adult animals whose condition markedly deteriorated during the hot summer period. Food supplementation with higher energy intake helped some individuals.

All animals formed 2 large groups which started to split after the first calving period. Thirteen animals, mainly weakened sub-adult individuals, died during the first year. A few adults died due to ingestion of different foreign objects including plastic bags, ropes, irrigation pipes and rubbish left over from of previous camel farms. Later the most common cause of mortality was traumatic injuries of males, which fought for positions in the social hierarchy around feeding spots. The redistribution of feeding sites to remote places reduced the contact time and consequently the chances for conflict between individual groups. A few cases of calves killed by dominant males were reported as well. Only 1 known foetus malposition case occurred during 4 years of observation.

Translocation of sick or wounded individuals was successfully carried out by darting the animals with mixture of 1 mg. kg^{-1} xylazine and 1 $mg.kg^{-1}$ ketamine, reversed with 1.5 ml antipamezole (ANTISEDAN) i.m. *pro toto*. This anaesthesia procedure was introduced after recurrent cases of capture myopathy and mortalities due to hyperthermia using ethorphine (IMOBILON) in hot summer months (8, 3). During the first 4 years the population has grown, reaching more than 200 individuals at the time of writing. Food supplementation with hay was important during summer months to prevent extensive damage on the vegetation and severe deterioration of body conditions in young, poorly adapted animals.

RESULTS

The project shows that Arabian oryx, even after a long time in captivity, can easily establish back into natural conditions. There are few health risks if the animals are separated from domestic live stock. The biggest challenge for reintroduction of animals back into free ranging in conditions in the UAE will be to find a suitable environment that is large enough to provide sufficient diverse vegetation for shade and grazing. Around 850 kg dry matter of food is required per animal per year, so it will be difficult to find places with suitable abundant vegetation for large groups of oryx.

Permanent food supplementation will be probably be necessary to avoid extensive overgrazing. Management guidelines accepted by all parties involved in Arabian oryx breeding will be essential in the future to coordinate the diversity of the population.

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DISINFECTION BYPRODUCT FORMATION ACCORDING TO TYPE OF DISINFECTANT USED

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ABSTRACT

Results of the Petrinja-Sisak water supply system safety testing for potentially harmful disinfection by-products collected over years are presented. They showed that the formation of Trihalomethanes (THMs) was significant only when gaseous chlorine was used in pretreatment and as the final disinfectant. Chlorine dioxide produced little THMs, and chlorite concentration detected was below guideline values. Preozonation generally decreased THMs formation from postchlorination, either chlorine or chlorine dioxide was used as secondary disinfectant.

Key words: chlorine; chlorine dioxide; disinfection byproducts; drinking water; ozone; THMs

INTRODUCTION

Drinking water must be such that its consumption or use does not endanger health. Disinfection is vital for preventing the spread of diseases caused by waterborne pathogens, but the use of chemical disinfectants usually results in the formation of by-products with potential health concerns. Chlorination is in most cases the primary method of disinfection and chlorine is one of the most commonly used disinfectants for water disinfection. The discovery that a large number of by-products are formed by the reaction of chlorine with naturally occurring organic materials (2) raised concern about potential health effects that might result from exposures to these compounds (3).

Chlorine dioxide is very different from elementary chlorine in its chemical structure as in its behaviour. The end products of chlorine dioxide reactions are chloride, chlorite and chlorate. The residual by-product chlorite is suspected to have health effects. Ozone is one of the most powerful oxidants available for potable water disinfection owing to hydroxyl radicals ($OH^{\cdot}$).

In the present study the results of a year-long monitoring of drinking water safety with the use of ozone, gaseous chlorine and chlorine dioxide on water treatment and disinfection are presented in relation to the formation of Trihalomethanes by-products, i.e. chlorites, associated with health risks.

MATERIAL AND METHODS

Natural surface waterflow of the Kupa River is used as a source by the water supply system Petrinja-Sisak. Raw water samples were collected from drinking water plant intake, and drinking water samples, conditioned and disinfected, were collected on the location in the city center.

In the first study period (1995–1997), the treatment of natural surface water input included, in addition to rough and fine mechanical purification, precipitation, sand filtration, only gaseous chlorine in pretreatment and in disinfection.

In the second period from 1998 on, the pretreatment with ozone as primary disinfectant was introduced and gaseous chlorine was used as a secondary disinfectant.

In the third period from 2004 on, gaseous chlorine was substituted by chlorine dioxide for disinfection and re-chlorination.

Determinations of physicochemical and bacteriologic parameters were done in accordance with the standard methods aimed for drinking water. To determine Trihalomethanes, the

Table 1. Mean values of Trihalomethanes and chlorite in drinking water according to the method of disinfection

Parameter (μg.l^{-1})	TCM	BDCM	CDBM	TBM	TTHM	Chlorite
raw water – Kupa river	0.24	0.15	0.20	0.16	0.54	0
prechlorination/chlorine	32.83	2.90	0.12	0.15	35.19	0
preozonation/chlorine	7.89	2.71	0.67	0.00	11.27	0
preozonation/chlorine dioxide	0	0	0	0	0	0.17

gas chromatography USEPA Method 551.1, was used. Chlorites were determined according the Ion chromatography EPA Method 300.1.

RESULTS AND DISCUSION

The results of Trihalomethanes and chlorite formation according to the three different models of disinfection are presented, as mean values of year-long monitoring, in Tab. 1.

Trihalomethanes (THMs) are the most prevalent group among disinfection byproducts formed during chlorination of natural surface waters. The Kupa River does not contribute to the drinking water input of THM with mean level of 0.54 μg.l^{-1}. However, an organic substance present in the Kupa River, with a mean TOC level of 3.90 mg.l^{-1} and mean humic acid level of 1.43 mg.l^{-1}, limited the use of chlorine on river water treatment. Results obtained during the first study period indicated all four THM compounds to be present in drinking water, as follows: chloroform (TCM) bromodichloromethane (BDCM); chlorodibromomethane (CDBM); and bromoform (TBM). The highest levels exceeding the maximal allowed concentration (MAC) by 67.24% were measured for TCM, which was most frequently present in study samples (93.31 %).

Alternative disinfectants such as ozone and chlorine dioxide can be used to prevent the formation of hazardous byproducts such as Trihalomethanes (1).

Studies conducted from 1998 on refer to the period when ozone was introduced as primary disinfectant. All THMs levels were below MAC. Results obtained have suggested the level of TCM to be reduced by 80% with the use of ozone on water conditioning.

Since 2004, when water disinfection with chlorine dioxide has been introduced, testing for the presence of THMs has showed that they were generally absent in study samples. As the chemical mixture of chlorine dioxide disinfection by-products, i.e. chlorites and chlorates are also declared as compounds posing a health risk, systematic chlorite measurements are warranted on water safety analysis. Their mean annual levels were below MAC.

In general it is best to remove as much organic matter as possible from water before disinfection will be applied. This can be achieved with the existing water treatment techniques.

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DEATH, FOLLOWING THE REGRESSION OF TRANSMISSIBLE VENEREAL TUMOR (TVT) IN A NIGERIAN LOCAL MALE DOG (MONGREL) TREATED WITH ONCOVIN (VINCRISTINE) (CASE REPORT)

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ABSTRACT

The possible effects of vincristine sulphate administration to a male dog which had Transmissible venereal tumour (TVT) were studied. A weekly intravenous (i. v.) injection of 0.025 mg.kg^{-1} vincristine sulphate was administered in the first 2 weeks and the dose halved for the next 4 weeks. Vitamin B-complex and 5% dextrose solution were also administered i. v. By the 5th week, significant reduction in the size and firmness of tumour as well as fluid exsudation were noticed. The dog later became anorexic and recumbent with persistent crying. Death followed 2 weeks after the end of the six-week period of treatment. The study indicates that vincristine sulphate is effective against canine TVT but death of the subject may occur afterwards, probably due to toxicosis.

Key words: canine transmissible venereal tumor; death; regression; vincristine sulphate

Running Head: ***Death in the resolution of Transmissible Venereal Tumour using Oncovin (Vincristine Sulphate)***

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INTRODUCTION

Transmissible veneral tumour (TVT) is a contagious veneral tumour of dogs, which has continued to be a serious problem around the world (11) especially in Nigeria (8). The condition has been reported to occur at same frequency in both male and female dogs (16) with higher frequencies occurring in stray and wild dogs, which exhibit indiscriminate sexual activity (4).

The neoplasm ranges from 5 mm to 10 cm, it may be pedunculated, modular or multilobulated with the superficial part commonly unclerated and inflamed and may be haemorrhage and infected (15). The initial growth of the tumour is rapid but slower later, with metastasis uncommon. When metastases occur, it is usually to the superficial inguinal lymph nodes, kidney, spleen, eye, brain, pituitary, skin and subcutis, mesenteric lymph nodes and peritoneum (5). The possibility of transmissible venereal of transmissible venereal tumour as a predisposing factor to urinary tract infection has been reported (1).

Various therapeutic methods that have been adopted in the management of the condition include surgery, diathermy, cryosurgery, irradiation and chemotherapy (7). Combination of anti-tumour drugs rather the use of single agent was reported as a more effective method in the treatment of TVT (3, 9, 13).

Oncovin (vincristine) is an alkaloid derived from the periwinkle plant (*Catharanthus roseus*) and exerts its antineoplastic effect by binding to tubulin and thus blocking mitosis (18).

Fig. 1. Shows the largest tumour (≈ 8 cm diameter) located at the pelvic region. It consists of 3 open wounds. This is the point at which treatment commenced

Fig. 2. A single large ulcer formed by the coalescing of the initial 3 open wounds from figure 1. This was taken a week into treatment

Fig. 3. A progressive in the size of tumour mass and open wounds. Picture taken 2nd week into treatment

Fig. 4. A large extent of healing has taken place. This is shown by the further reduction in size of the wound and tumour pass. Picture taken 5th week into treatment

Vincristine is rapidly taken up into cells and its cytotoxicity correlates directly with its intracellular concentration (14). Commonly encountered toxicoses include vomiting, anorexia, myelosuppression, weakness, parethesia and decreased reflexes (18). It was suggested that when anorexia is prolonged, dose reduction should be recommended for subsequent treatment (18). However, excellent results have been obtained in the use of vincristine in the treatment of lymphomas and sarcomas like transmissible venereal tumour (2).

CASE HISTORY

A male local dog (Whisky) about 2 years old weighing 16.4 kg was presented in February, 2004 with a history of lumps on the pelvic region. The growths were said to have started as a single tiny growth adjacent to the base of the scrotal skin. On examination, the lumps were found to be solid masses of varying sizes with the largest (≈ 8 cm diameter) ulcerated and adjacent to the scrotum (Fig. 1). The condition was clinically diagnosed as transmissible venereal tumor with metastases to the skin and subcutis of the abdominal and thoracic regions.

MANAGEMENT

1 mg pack of lyophilized vincristine sulphate (Vinstin; Neon Antibiotics, PVT, India) was reconstituted with 10 ml of diluents provided by the manufacturer to obtain 0.1 mg vincristine sulphate.ml^{-1}. A weekly intravenous (i. v.) injection of the drug at 0.025 $mg.kg^{-1}$ was carried out initially for the first 2 weeks after which subsequent doses for the next 4 weeks were halved. Vitamin B-complex injection as well as 5 % dextrose i. v. was administered both to stimulate the appetite and reduce the effects of dehydration. The open wound was also cleaned daily with an antiseptic solution and the dog was allowed to rest in a fly proof apartment.

RESULTS

The dog remained active throughout the first five week of treatment. Obvious and progressive signs of healing characterized by reductions in the firmness of tumour masses, size of the open wound and fluid exsudation were noticed. Figures 1–4 show some of the gross changes

observed in the size of the tumour during healing. By the 4th week of treatment also, the smaller lumps located in the abdominal and thoracic regions regressed totally. However, by the 6th week of treatment, the dog developed a progressive anorexia and dehydration. Episodes of vomiting and infrequent diarrhoea were also noticed. The dog later became recumbent with generalized stiffening of muscles and persistent crying and death within 48 hours following crying was observed.

DISCUSSION AND CONCLUSION

Reports on the various drugs judiciously used in cancer therapy have shown their efficiency and safety in both humans and animals. Administration of anti-tumour drugs gave better results, reduced cost of therapy, produced minimal toxic side effect, minimal or no stress on the animal as well as rare post treatment recurrence (12, 6, 7, 2, 3, 8). Vincristine sulpathe (Oncovin) is an alkaloid derived from the periwinkle plant (*Catharanthus roseus*) and it is a mitotic inhibitor, destroy the mitotic spindlesss of the cell and prevent further cell division (12).

In the various report by Hess *et al.* (7), (8, 13) an encouraging result have been achieved by treating TVT with cyclophosphamides and methorexate as a single and/or combine treatments and therefore the results in this study are similar to earlier report that administration of vincristine sulphate alone, without necessarily combining with the common alkylating agent (cyclophosphamide) was effective against canine TVT (17).

The anorexia, vomiting and recumbency etc. observed were progressively linked and typical of vincristine toxicosis (18). The anorexia and vomiting will lead to body weakness and the recumbency will result into muscle atrophy and wasting (10).

Vincristine was also reported to cause anaemia and leukopenia (13, 17,) as well as myelosuppression and depressed reflexes (18). The cumulative effect of these factors coupled with the long-standing duration of the tumor on the dog are suggested as the possible cause for the observed death. However, a more detailed investigation may be necessary to confirm that other factors are not involved.

The study indicates that the administration of vincristine alone will cause regression of canine TVT. However, there is need for effective management of the patient during and after treatment to avoid death either from toxicosis or other possible sources.

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THE SPERMIOGRAM OF MALE WISTAR RATS TREATED WITH AQUEOUS LEAF EXTRACT OF *Vernonia amygdalina*

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ABSTRACT

The reproductive implications of the aqueous leaf extract of *Vernonia amygdalina* were studied using twelve male albino rats (Wistar strain). Two experimental groups (A and B) were treated with 500 mg.kg^{-1} body weight and 250 mg.kg^{-1} body weight respectively for 15 days and the control group C were not treated with the extract. The spermatozoa from the caudal epididymides were then studied in these three groups. It was observed that the mobility of spermatozoa from groups A and B with percentage values of 34.00 ± 2.28 and 60.00 ± 0.00 respectively were significantly lower (P > 0.05) than for group C which had 92.50 ± 0.00. The percentage livability of the sperm cells were also lower in groups A and B with 92.00 ± 0.54 and 95.20 ± 0.75 respectively than for the control of group with value of C 98.00 concentration of the experimental groups 53.20 ± 1.49 for group B) were also significantly lower (P > 0.05) than for the control group (C 88.50 ± 0.00). The histopathology of the testes revealed hypoplasia of the seminiferous tubules in groups A and B as compared to the normal somniferous tubules in the control group (C). It can be concluded, therefore, that the uncontrolled use of *Vernonia amygdalina* have an adverse effect on the spermiogram and spermatozoa/morphology of the intact male Wistar rats.

Key words: spermiogram; *Vernonia amygdalina; Wistar rats*

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INTRODUCTION

Vernonia amygadalina is a shrub of 2–5 m with petiolate leaf of about 6 mm in diameter and elliptic shape (Compositae). The leaves are green with a characteristic odour and a bitter taste. It is propagated by cutting. There are about 200 species of *Vernonia* and it grows under a range of ecological zones in Africa (11).

The use of medicinal herbs have increased over the past few years and research interest has focused on various herbs that possess anti-tumour or immune stimulating properties that may be useful adjuncts in helping to reduce the risk of cancer (3). This was why Nwude (8) said that s research into the efficacy of herbs used in traditional veterinary practice would be useful in establishing standard dosages for herbal preparations and to investigate their toxicity. One of such herbs is *Vernonia amygdalina.*

Vernonia amygdalina is commonly known as bitter leaf. It is known as ewuro in Yoruba and onugbu in Ebonyi State, and chusar doki in Hausa land. Traditionally the leaves of this herb are consumed in many parts of Nigeria as vegetable. The leaves and stems are also chewed to cure stomach aches, stimulate the digestive system. It can also be used as insecticide. The aqueous extract is used as tonic drink for prevention of

certain illnesses for example as anti-helminthiasis, as laxative anti-malaria and trypanocide (1, 14). A cold infusion of the root bark is used to treat bilharzias (4). The vegetable when incorporated in the diet of women may prevent or delay the onset of breast cancer.

The phytochemisry of this herb have been studies to some extent. According to Phigashi *et al.* (11) and Jisaka *et al.* (7) reported that it is made up of several stigmastanetype saponins, such as vernonioside A, B1, A2, A3, B2, D3, A4, and C. In 2004, Akindahunsi and Salawu reported that the sundried leaves of this herb contain saponins, tannins and minerals, like iron, sinc, magnesium, sodium, potassium, calcium and phosphorus, Igile *et al.* (5) using coupled oxidation of B-carotene linoleic and demonstrated the anti-oxidant activities of luteolin-o-β-glucoside flavonoid compounds present in the leaves of *Vernonia amygdalina.* It was also discovered that the leaves contain vernodaline and vernolide which were demonstrated by Jisaka *et al.* (7) to elicit antitumoural activity in leukaemia cell P-388 and C 1210 with IC 50 values of 0.11 and 0.17 $\mu g.ml^{-1}$ for vernodaline and 0.13 and 0.11 $\mu g.ml^{-1}$ for vernolide. Extracts from the herb have cell growth inhibitory effects in prostate cancer cell 1ms (PC-3) and no effect on human peripheral blood mononuclear cells (PBMC) and some peptides from the aqueous extracts of the leaves are potent inhibitors of mitogen-activated proteinkinase (MAPKS) which are crucial for breast tumour growth and also inhibit DNA synthesis in a breast cancer cell line (6). The haemolytic effect of saponin extract of *V. amygdalina* was reported by Oboh (9).

It was discovered that there was no report on the effect of *Vernonia amygdalina* extract in any form either as aqueons or saponin on the reproductive functions of the male knowing that this herb is consumed by both male and female, hence this study was carried out to evaluate the sperm characteristics and morphology of the male Wistar rats that have undergone treatment with varying doses of the aqueous *V. amygdalina.* Since fertility in the male animal depend on the viability and concentration of the sperm cells produced by the testes, the outcome of this study will constitute a source of baseline data and basis for advising the ethnomedical practitioner and the general public on the usage of this herb.

MATERIALS AND METHODS

Fifteen sexually male albino rats (Wistar strains) were used for this study. The rats were housed in the experimental animal unit (EAU) of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The rats were fed *ad libitum* with commercially prepared rat feeds made up of 21 % protein, 3.5 % fat, 6 % fiber, 0.8 % phosphorus (Ladokun Feeds Limited, Ibadan, Nigeria) and given fresh water. They were kept in cages which have wood shaven as bedding and cleaned once a week. The feeds and water were provided *ad libitum* in earthen troughs. Three cages were used and each measured about 18 by 12 inches. The rats were in groups of 5, 5 and 5 in each cage marked A, B and C respectively under the same environmental and management conditions.

The aqueous extract was prepared using 400 ml of distilled water to 200 *g* of fresh *Vernonia amygdalina* leaves. The concentration of the extract was determined using this formula.

$$\text{Concentration} = \frac{\text{Weight of leaves g}}{\text{Quantity of water ml}}$$

And the actual dose to be administered was obtained using this formula:

$$\text{Dose (ml)} = \frac{\text{Dosage mg.kg}^{-1} \times \text{Body weight (kg)}}{\text{Concentration mg.ml}^{-1}}$$

After 2 weeks of stabilization the rats in groups A and B were given aqueous extract of *Vernonia amygdalina* leaf at 500 $mg.kg^{-1}$ body weight and 250 $mg.kg^{-1}$ body weight respectively *per os* for a 5 days using needle and canular to introduce the extract directly into the stomach through the oesophagus. Group C rats were the control with no administration of *Vernonia amygdalina* extract.

The rats were then anaesthetized by placing them into a glass chamber containing cotton wool soaked in chloroform till they lost consciousness. The testicles were then removed through a lower abdominal incision. The testes were then separated from the epididymis with the scalped blade. Sperm cells were sucked into a pre-warmed (37 °C) Pasteur pipette from the caudal epididymis. It was flushed with 2–3 drops of 2.9 % sodium citrate self kept at 37 °C. Smears were prepared from these samples and strained with wells and Awa stain for morphological studies and eosin and nigrosin stain for live/dead ratio (16). Half of the spermatozoal samples collected were mixed with 0.5 ml of 2.9% sodium citrate solution (7 °C) for head forward unidirectional progressive motility. These were studied at ×40 magnifications of the microscope (16).

The mean percentages and standard error of mean were calculated for motility, live/dead ratio, Spermatozoal concentration and morphological studies. ANOVA (Analysis of variance) was used to establish any significant difference in all these parameters (15).

RESULTS

It was observed that the groups of rats that had the highest dose of the aqueous extract (500 $mg.kg^{-1}$ body weight and those of 250 $mg.kg^{-1}$ body weight had their percentage motility 34.00 ± 2.8 and 60.00 ± 0.00 % respectively) significantly lower ($P < 0.05$) than that of the control group (92.5 ± 0.00 %). The higher the dosage the higher the gravity of the effect of the extract on the motility of the sperm cells (Table 1).

The percentage livability (live-dead ratio) of the sperm cells was 92.0 ± 0.5, 95.2 ± 0.75, and 98.0 ± 0.00 % for the doses of 500 $mg.kg^{-1}$, 250 $mg.kg^{-1}$ and the control group respectively. There was a reduction on the number of the live spermatozoa with 98.00 ± 0.05 % in the control group compared to the groups A & B with 92.00 ± 0.5 and 95.20 ± 0.75 % respectively.

The sperm cell concentration 53.20×10^6 cells.ml^{-1} at group A and the count of 67.40×10^6 cells.ml^{-1} were lower than the count 88.50×10^6 cells.ml^{-1} in the control group and this difference is significant ($P < 0.05$) and this is confirmed by the histopathologic slide of the testes (Figs. 1, 2) where there was hypoplasia of the seminiferous tubules of the testes of rats in group A.

The morphological characteristic of the sperm cells in all the three groups of rats are as shown on Table 2, the following abnormalities were noticed both in the control group and the treated groups A & B; head less tail, rudimentary tail, curved mid-piece, curved tail, coiled tail, looped tail, bent mid piece tailless head and bent tail, but the percentage of abnormal sperm bells were highest (11.05 %) in the group A treated rats, followed by those (8.5 %) treated in group B. The control group was the lowest (6.45 %).

DISCUSSION AND CONLUSION

The study showed that the percentage motility of the control experiment (group C) rats 92.5 % was higher than 76.00 ± 2.40 % reported by Oyeyemi *et al.* (12) for same species of rats. These values are significantly higher ($P < 0.05$) than motility values of 34.00 % for group A rates (treated with 500 mg.kg^{-1} body weight) and 60.00 % for group B rates 250 mg.kg^{-1} body weight). Although the percentage livability of sperm for group A (92.00 %) was lower than group B (95.20 %) and also lower than group C value (98.00 %). However, livability percentages in the three groups are higher than what was recorded (88.00 ± 3.39 %) by Oyeyemi *et al.* (12) for male Wistar rats without any treatment.

The sperm count (concentration)of group C 88.50×10^6 cells.ml^{-1} was significantly higher ($P < 0.05$) than group A value (67.40×10^6 cells.ml^{-1}) and this difference was significant ($P < 0.05$) as evidenced by the hypoplasia of the seminiferous tubules in the histopathological slides of the testes of rats from group A indicated that very high dozes (250–500 mg.kg^{-1} body weight of aqueous leaf extract of *V. amygdalina* for a long time like 15 days will have adverse effect on the sperm concentration. The morphological abnormalities also increased as the concentration of the extract increased as observed in this study.

It can be therefore concluded that dosages between 250–500 mg.kg^{-1} body weight of the aqueous leaf extract of *Vernonia amygdalina* for as long as 15 days will have a deleterious effects on the reproductive functions of the male Wistar rat and so it is advised the continuous usage of aqueous leaf extracts of *V. amygdalina* should be with caution.

Table 1. Sperm characteristics of Wistar rats in groups A + B and C (± SEM)

Identification	Motility (%)	Live/dead ratio Livability (%)	Sperm count × 10^6 cells.ml^{-1} (concentration)
A 500 mg.kg^{-1} bw	34.00 ± 2.8	92.00 ± 0.54	53.20 ± 0.53
B 250 mg.kg^{-1} bw	60.00 ± 0.00	95.20 ± 0.75	67.40 ± 1.49
C No treatment	92.50 ± 0.00	98.00 ± 0.00	88.50 ± 0.00

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Table 2. Sperm morphology of Wistar Rats in Groups A + B and C (± SEM)

Identification	Headless tail (%)	Rudimentary tail (%)	Curved Mid-Piece (%)	Curve tail (%)	Looped tail (%)	Bent mid-piece (%)	Tail-less head (%)	Bent tail (%)	Total abnormal (%)	Total normal (%)	Total cells counted (%)
A	26 (1.37)	27 (1.42)	27 (1.42)	4 (0.021)	4 (0.021)	27 (1.42)	22 (1.16)	38 (2.01)	209 (11.05)	1681 (88.94)	1890 (100)
B	20 (1.01)	24 (1.21)	25 (1.26)	0	2 (0.10)	20 (1.01)	16 (0.80)	32 (1.61)	169 (8.53)	1811 (91.46)	1980 (1980)
C	6 (0.74)	5 (0.62	10 (1.24)	0	0	6 (0.74)	5 (0.62)	11 (1.36)	52 (6.45)	753 (93.54)	805 (100)

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THE EFFECTS OF THE AQUEOUS EXTRACT OF "UGU" (*Telfairia occidentalis)* LEAVES ON THE TESTIS AND SPERMATOZOA CHARACTERISTICS IN THE MALE ALBINO RAT (WISTAR STRAIN).

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ABSTRACT

The effects of the aqueous extract of "ugu" (*Telfairia occidentalis*) leaves on some blood parameters, testis and spermatozoa of male albino rats (Wistar strain) were investigated. 500 mg.kg^{-1} and 1 000 mg.kg^{-1} of the aqueous extract were administered once daily orally to two groups (A and B) respectively for 14 days. Group C, which served as control received 0.2 ml of distilled water daily. Blood samples were evaluated for haematology.

The extract at separate concentrations caused significant increase ($p < 0.05$) in the levels of packed cell volume, haemoglobin and erythrocyte.The spermiogram and testicular histology were also evaluated. Results revealed a significant decrease ($p < 0.05$) between experimental group B and control for spermatozoa concentration. The motility for experimental groups A and B were lower ($p > 0.05$) compared with control while the control value for percentage livability was lower ($p > 0.05$) when compared with experimental groups A and B. Testicular histology showed mild to severe testicular degeneration and interstitial oedema of seminiferous tubules. Our findings suggest a boosting effect on blood parameters by the extract but deleterious effect on seminal characteristics.

Key words: aqueous extract; rat; spermatozoa; testis; "ugu"; *Telfairia occidentalis*

INTRODUCTION

Ugu plant (*Telfairia occidentalis)* is one of the commonest leaf vegetables consumed in Nigeria. *Telfairia occidentalis* belong to a larger family known as Cucurbitaceae. The cucurbitaceas are reported to have been associated with man since 12, 000 BC (9) and are cultivated across lowland humid tropics of West Africa, mainly for their nutritional value (3). Common examples of plants in this family are *Cucumber, Watermelon, Pumpkin, Squash and Melon* (12). New cultivars, which are better, being elongated and seedless seems to have replaced earlier forms of the plants (15).

The fruits of *T. occidentalis* are inedible but the leaves and seeds (without hulls) contain 21.2 % and 35.7 % protein respectively and are edible both to man and animals (14,11). Christian, (7) reported that oil extracted from pumpkin has a high degree of unsaturation compared to palm oil and suggested it as a better alternative for cooking and margarine production. The pharmacological importances of this family of plants are ample. Momocharin and luffaculin are two abortifacient proteins isolated from the plants from this family with ribosome-inhibiting properties, which have been used to induce second trimester abortion (14). A third protein is trichosanthin (from *Trichosanthes species*), which has demonstrated the capacity to inhibit the multiplication of human immunodeficiency virus

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(HIV) within lymphocytic and phagocytic cells (14). Similarly, the aqueous extract of *T. occidentalis* has been shown to be hepatoprotective against garlic-induced oxidative stress (16) while its ethanolic extract have demonstrated hypoglycaemic properties both in normoglycaemic and alloxan-induced diabetic rats (17).

Though the administration of *T. occidentalis* seed oil have lowered plasma cholesterol level in the rat, artheriosclerosis and reducing other degeneration changes have been detected in several organs including the heart, liver and kidney (2). Despite the fact that "ugu" is widely consumed by man and his animals in the tropical regions and the leaf and seed are rich in protein, there is limited or no information on the effect of this plant on seminal characteristics. This paper therefore reports the effects of the aqueous leaf extract of "ugu" plant on blood parameters, spermiogram and testicular histology of experimental rats.

MATERIALS AND METHODS

Fifteen male albino rats (Wistar strain) were obtained from the Animal house of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria for the study. The rats weighed between 90–80 grams. They were kept in rat cages within the Experimental animal unit and fed with rat cubes (Ladokun Livestock Feeds, Nigeria Ltd.). They were allowed free access to clean fresh water in bottles *ad libitum.* They were allowed to stabilize for a period of 14 days following which they were randomly divided into three groups (A, B, and C) of five rats each. Group C being the control group Fresh leaves of Ugu (*Telfairia occidentalis*) were collected from the Teaching and Research Farm of the University of Ibadan, Nigeria. The leaves were authenticated at the herbarium of the Botany Department of same University. The leaves were washed in distilled water and allowed to dry on the bench at room temperature for 5 days. They were then chopped into smaller bits.

12.5 grams of the chopped form was thoroughly blended in 24 mls of distilled water. The aqueous extract was obtained following filtration of the blended mixture into a conical flask using filter paper. The concentration of the filtrate (aqueous extract) was 500 $mg.ml^{-1}$ and it was maintained throughout the study at refrigeration temperature. Stomach canula was used to administer the filtrate to the rats used in the study. Group A rats received 500$mg.kg^{-1}$ body weight while Group B received 1000$mg.kg^{-1}$ body weight during 14 days.

The rats were anaesthetized with chloroform for about 5 minutes. Blood was collected through the medial canthus of the eye into heparinized bottles for haematology. Determination of haemoglobin concentration was as described by Schalm *et al.,* (17) using the cyanomethaemoglobin method. The packed cell volume (PCV) was estimated using the conventional method of filling capillary tubes with blood and centrifuging (17). Red blood cell count was determined via haemocytometer method described by Coles, (8).

The anaesthetized rats were also placed on dorsal recumbency and the testicles were surgically removed through a lower abdominal incision. The epididymides were trimmed off the testes and sample was collected from the cauda through an incision made with a scalpel using a Pasteur pipette.

Semen examination to evaluate spermatozoa characteristics such as motility, live-dead ratio, concentration and morphology were done using methods described by Zemjanis (18). In these methods, motility was evaluated with one drop each of semen sample and 2.9 % sodium citrate buffer under a cover-slip and viewed under × 40 of microscope. Semen smears prepared were stained with Wells and Awa stain for morphology and Eosin-nigrosin for live-dead ratio. The spermatozoa concentration was also evaluated using the improved Neubar chamber method of counting.

Testes from all animals were fixed in 10 % buffered formalin in labelLed bottles. They were processed routinely by embedding in wax (paraffin). 5 µm Thick sections 5 µm were then cut, stained with haematoxylin and eosin (13) and examined under the microscope.

Data collected were subjected to Student-*t*-statistic with level of significance taken at 5 % (10).

RESULTS

The changes in haematological parameters in the study are shown in Table 1.

The extract caused significant increase ($p < 0.05$) in the levels of PCV and Hb concentration at 500 $mg.kg^{-1}$ while a similar increase in Rbc concentration was caused by the 1 000 $mg.kg^{-1}$ dosage. An increase though not significant ($p > 0.05$) was observed for PCV at 1 000 $mg.kg^{-1}$ and RBC at 500 $mg.kg^{-1}$. There was also a significant increase ($p < 0.05$) in Hb concentration with 1 000 $mg.kg^{-1}$ dosage.

The changes in spermatozoa characteristics of the rats during the study are shown in Table 2. The spermatozoa motilily for both groups A and B rats are lower ($p > 0.05$) than for control group. Similarly, the percentage livability for groups A and B were higher ($p > 0.05$) than for control group. The spermatozoa concentration for group A was higher ($p > 0.05$) than for control group while value for group B was lowest. The difference between groups B and C was significant ($p < 0.05$). The difference in the percentages of spermatozoa morphological abnormalities for all three groups is shown in Table 3. The differences are similar and not significant ($p > 0.05$).

Histopathological examination of sections of the seminiferous tubules showed moderate degeneration and interstitial edema, which are more prominent with group B (1 000 $mg.kg^{-1}$ dosage) rats (Figs. 1, 2 and 3).

Table 1. Effects of graded doses of aqueous extract of "ugu" plant on packed cell volume, Haemoglobin and erythrocyte concentration of the rats

Group(s)	PCV %	Hb Concentration (mg%)	Rbc X 10 12/L
A	43.6 ± 0.66[a]	13.66 ± 0.29[a]	9.15 ± 0.48[b]
B	40.8 ±1.02[b]	14.38 ±0.34[a]	10.87 ± 0.15[a]
C	37.25 ±0.75[b]	12.47 ± 0.26[b]	8.99 ±0.24[b]

a, b – numbers differently superscripted in each column are significantly different ($p < 0.05$)

Table 2. Effects of graded doses of aqueous extract of "ugu" plant on motility, on percentage liability and spermotozoa concentration of the rats

Group(s)	Motility (%)	Percentage livability (%)	Spermatozoa concentration (×10b cells.ml^{-1})
A	74.00 ± 8.12[b]	94.20 ± 8.12[b]	81.20 ± 2.71[b]
B	76.00 ± 5.10[b]	92.60 ± 2.29[b]	59.00 ± 2.37[a]
C	86.25 ± 3.75[b]	92.00 ± 2.86[b]	70.25 ± 4.21[b]

a, b – numbers differently superscripted in each column are significantly different ($p < 0.05$)

Table 3. Effects of graded doses of the aqueous extract of "ugu" plant on spermatozoa morphological abnormalities

Group(s)	Total abnormal cells	Total normal cells	Total cells counted
A	203 (10%)[b]	1827[b]	2030
B	231 (11.6%)[b]	1759[b]	1990
C	177(10.86%)[b]	1458[b]	1635

a, b – numbers differently superscripted in each column are significantly different ($p < 0.05$)

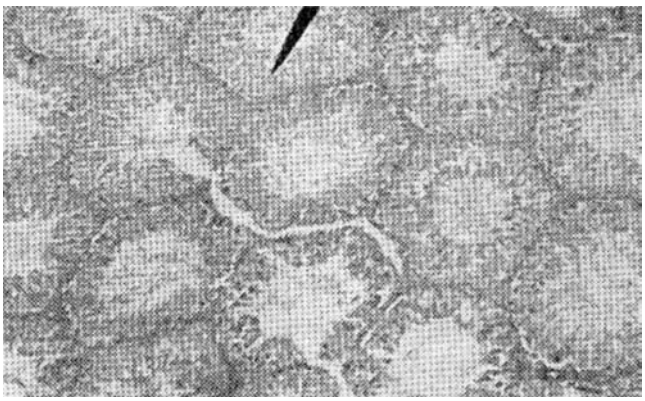

Fig. 1. Photomicrograph of seminiferous tubule of group A rat: moderate focal interstitial oedema in the seminiferous tubules (× 100)

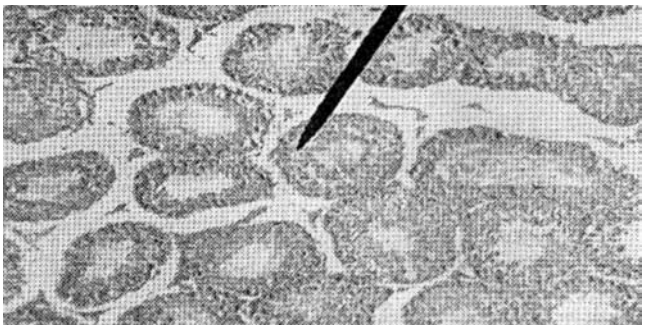

Fig. 2. Photomicrograph of seminiferous tubule of group B rat: hypoplasia and marked focal areas of interstitial oedema of the seminiferous tubules (× 100)

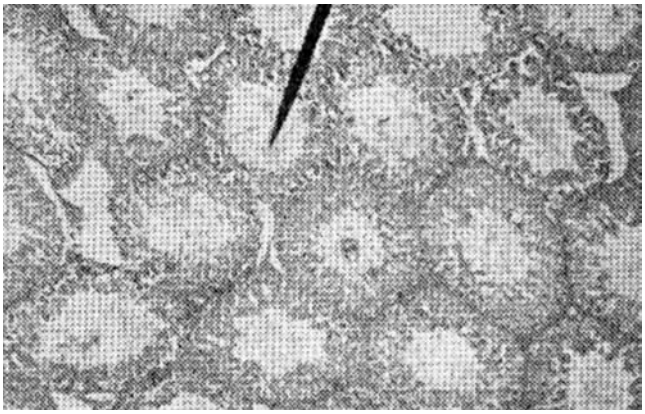

Fig. 3. Photomicrograph of seminiferous tubule of group C rat: seminiferous tubule of control rat (× 100)

DISCUSSION

The present study shows that the aqueous extract of "ugu" plant (*Telfairia occidentalis*) produced increase in the concentration of the haematological parameters evaluated i.e. packed cell volume, haemoglobin concentration and erythrocytes. This suggests that the aqueous extract of this plant possesses haematinic properties as reported by some workers (1). This haemopoietic effect may be due to the high content of different minerals in the leaves of the plant (5) The aqueous extract then could be a helpful substitute in cases of blood shortage or other conditions which places high demand on the blood forming system of the mammalian body such as pregnancy.

The semen parameters investigated in this study occupies a position of relevance in male fertility studies. The histological integrity of the testis is fundamental to the production of fertile spermatozoa. Any factor(s), which damages the testicles such as infectious, toxic agents, malnutrition or heat, will result in the production of subfertile spermatozoa.

The motility of sperm cells is important for fertilization since only sperm cells with progressive motility are involved. The aqueous extract of Ugu plant produced reduced motility of spermatozoa both at 500 $mg.kg^{-1}$ and 1 000 $mg.kg^{-1}$ dosage. The increase in livability of spermatozoa was very slight, however the 1 000 $mg.kg^{-1}$ dosage of the extract significantly reduced the spermatozoa concentration. This reduction in spermatozoa quality may be subject to the degenerative changes caused by the extract in the seminiferous epithelium and may be similar to the type reported in several organs by Ajayi *et al.* (2). The aetiology of this pathology have been traced to the presence of alkaloids (5), which have been observed with *Telfairia occidentalis* (4). These alkaloids are bioactivated to release reactive metabolites, which bind to cell molecules and cross-link DNA to cause cellular damage (6).

In conclusion, the aqueous extract of *Telfairia occidentalis* from this study possess haematinic properties with adverse effects on the testes and spermatozoa integrity.

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GONADOTROPIN TREATMENT PREVENTS SEASONAL INFERTILITY IN THE SOW (A Short Communication)

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ABSTRACT

The study investigated the reproductive performance of weaned sows in 7 large Hungarian pig breeding units during hot summer season. Weaned sows of parity 2–7 (a total of n = 14 892) were evaluated in two groups in 8 replicates: *Group 1* (n = 5 329): the sows were treated subcutaneously with 400 IU of Gonadotropinum sericum (pregnant mare serum gonadotropin, PMSG [eCG]) and 200 IU of Gonadotropinum chorionicum (human choriongonadotropin, HCG [hCG]) one day after weaning. *Group 2* (n = 5 001): the sows were treated as the sows of Group 1 and received additionally 1 g vitamin E as top dressing one day after weaning. *Group 3* (n = 4 562): the sows received subcutaneously 4 ml of physiological saline solution one day after weaning. Weaning to service intervals, regular and irregular returns to service, farrowing rates and subsequent litter sizes were evaluated. There were no significant differences in weaning to service intervals and regular returns to service between the sows from Groups 1 and 2. Sows from Group 3 had longer ($P < 0.001$) weaning to service intervals and more ($P < 0.001$) regular returns to service during hot weather periods compared with the sows from Groups 1 and two. Irregular returns to service were low and showed no significant differences between the groups. Farrowing rates and litter sizes were significantly ($P < 0.01$) better in Groups 1 and 2 compared with Group 3.

Key words: gonadotropin; reproduction; seasonal infertility; sow

INTRODUCTION

Seasonal infertility is defined as a decrease in fertility during hot summer months and lower subsequent reproductive performance (4). An increase in return to estrus and decrease in farrowing rates and litter sizes belong to the typical clinical signs of seasonal infertility (8, 1, 4, 5). It has been speculated (4) that suppressed gonadotropin secretion and ovarian activity are involved in seasonal infertility.

According to Hurtgen and Leman (6), conception losses are highest when females are heat stressed around the time of conception and implantation and manifest as regular returns to oestrus with total embryonic loss, or smaller litter sizes if embryonic mortality is limited. The same authors demonstrated that elevated temperatures significantly decreased ovulation rates. An USA study found an increase in weaning to service intervals and anoestrus during hot summer weather (4). Other authors (5) observed increased embryonic mortality when sows were exposed to high ambient temperatures up to 3 weeks after mating.

In order to prevent seasonal infertility, it has been suggested that exogenous gonadotropins should be injected at weaning during hot weather periods (4, 1, 3). According to a Hungarian study the administration of vitamin E at weaning decreased regular returns to oestrus during summer season (2).

The present study was conducted to prove the effect of exogenous gonadotropins with or without vitamin E supplementation on summer infertility in Hungarian sows.

MATERIALS AND METHODS

The study focused on investigation of the reproductive performance of weaned sows in 7 large Hungarian pig breeding units during summer season focusing on hot periods when the daily mean temperature exceeded 33 °C.

The sows were F1 or F2 genetic lines of Landrace x Large White, mated to Duroc or Landrace boars. The sows were kept in identical gestation and farrowing crates. Weaning took place at 4 weeks of lactation (27.8 ± 1.4 d).

Replacement gilts were introduced from the nucleus herd at between 140 and 150 days of age. The annual replacement rate in the period of 2001–2006 varied between 39 % and 48 %. The animals were bred at the first observed standing oestrus.

The standard farm management involved: group rotation of 30 females, double artificial insemination, and culling after parity 7.

Sows were kept in high-investment facilities according to the requirements of the intensive breeding enterprises of the early 1990s (farrowing crates, individual gestation crates, "eros" centre). The sows were artificially inseminated (AI) at their 1st post-weaning oestrus. After positive pregnancy diagnosis, they were moved into gestation barns. On day 110 of pregnancy the sows were transferred to farrowing houses and stayed there for the whole lactation of 3 weeks. Sow mortality rates of 6.7 % to 8.1 % *per annum* had been recorded in this herd during the previous years (2001–2006).

The unit had a pre-trial (2005) annual culling rate of 41.3 %. The pre-trial investigations revealed a high incidence (> 15 % of the sow inventory) of urogenital bacterial diseases accompanied by vulval discharges. Bacteriological examination of vulval discharges detected the presence of either *Clostridium* spp., *Actinobacillus suis, Klebsiella* spp., *Proteus* spp., (and) Streptococci, Staphylococci, *Erysipelothrix rhusiopathiae,* or *Escherichia coli*.

The sows were fed as follows:

- from AI to day 89 of gestation 2 kg/sow/d, and from day 90 to 110 of pregnancy, 3 kg/sow/d of a commercial gestation ration containing 12.2 megajoul digestible energy (MJ DE)/kg, 125 g/kg crude protein, 6.5 g/kg lysine, 8 g/kg calcium, and 6 g/kg phosphorus,
- from 110 d of pregnancy to parturition 3 kg/sow/d of lactating diet. On the day of parturition, 1 kg of feed was given. Beginning from the day 2 after parturition, the sows were fed *ad libitum* the same lactating diet, containing 13.0 MJ DE/kg, 180 g/kg crude protein, 10 g/kg lysine, 8 g/kg calcium, and 6 g/kg phosphorus,
- from weaning to mating the sows received *ad libitum* the same lactating diet, and 500 g potato starch / sow/d ("flushing").

Weaned sows of parity 2–7 (a total of n = 11 892) were evaluated in two groups in 8 replicates:

Group 1 (n = 4 329): the sows were administered subcutaneously 400 IU of Gonadotropinum sericum (pregnant mare serum gonadotropin, PMSG [eCG]) and 200 IU of Gonadotropinum chorionicum (human choriongonadotropin, HCG [hCG]) one day after weaning.

Group 2 (n = 4 001): the sows were treated as the sows in Group 1 and received additionally 1 g vitamin E as top dressing one day after weaning.

Group 3 (n = 3 562): The sows were administered subcutaneously 4 ml of physiological saline solution one day after weaning.

Weaning to service intervals, regular and irregular returns to service, farrowing rates, and subsequent litter sizes were evaluated.

Reproductive records were collected from individual sow cards.

"Non returning" females were subjected to transrectal pregnancy testing on days 21–25 post-insemination using an Aloka 500 V ultrasound (Aloka Co. Tokyo, Japan) with 5.0 and 7.5 MHz linear-array transducers fitted to fixed angle PVC adapters (transrectal stabilising rod).

The following parameters were evaluated:

- Weaning to service intervals
- Regular returns to service (days 18 to 25 and 37 to 44 post insemination)
- Irregular returns to service
- Farrowing rates
- Total litter sizes

Reproductive performance parameters were described using descriptive statistics. General linear model (GLM, Statistical Analytical System [SAS], 1989, Cary, North Carolina, USA) procedures were used to compare litter sizes. Variances of the means were compared by F-ratio testing and proportional data were compared using χ^2 analyses. A parity effect was included to account for variation due to differing maturity level of sows, and the sow genetics effect (F1 or F2) was considered to remove variations due to maternal heterosis.

RESULTS

There were no significant differences in weaning to service intervals and regular returns to service between the sows from Groups 1 and 2. Sows from Group 3 had longer ($P < 0.001$) weaning to service intervals and more ($P < 0.001$) regular returns to service during hot weather periods compared with the sows from Groups 1 and 2. Irregular returns to service were low and showed no significant differences between the groups. Farrowing rates and litter sizes were significantly ($P < 0.01$) better in Groups 1 and 2 compared with the Group 3 (Table 1).

DISCUSSION

The results presented showed a significant improvement in fertility of gonadotropin treated sows during high temperature periods compared with the control sows. However we observed no significant effect of vitamin E supplementation in this study.

The present study supports the published data that PMSG and HCG treatment at weaning during hot summer weather periods provides significant advantage in reproductive performance of sows. Peters and Pitt (8) and Almond and Bilkei (1) reported low reproductive performance in non treated sows during hot summer periods which resembled that observed in our control sows.

Table 1. The effect of gonadotropin (GN) or gonadotropin and vitamin E (GONE) treatment one day after weaning during high temperatures on reproductive performance of sows. Weaning to service intervals (WSI), subsequent regular returns to service (RRS), subsequent farrowing rates (FR) and subsequent born litter sizes (LS, excluding mummies and prepartal stillbirths, including intrapartal stillborn piglets) were evaluated

Parameter											
WSI d ± SE			RRS % ± SE			FR % ± SE			LS % ± SE		
Control	GN	GNE	Control	GN	GNE	Control	GN	GNE	Control	GN	GNE
9.8 ±1.7 c	6.1 ±1.4 d	6.0 ±1.2 d	6.2 ±1.3c	2.6 ±1.6 d	2.2 ±1.5 d	70.2 ±5.7 a	86.9 ±2.5 b	87.4 ±2.1 b	9.4 ±1.2 a	11.9 ±1.0 b	11.9 ±1.1 b

Significance within a parameter between the groups: a, b – $P < 0.01$; c, d – $P < 0.001$

GN: The sows were treated subcutaneously with 400 IU of Gonadotropinum sericum (pregnant mare serum gonadotropin, PMSG [eCG]) and 200 IU of Gonadotropinum chorionicum (human choriongonadotropin, HCG [hCG]) (Suidan®, Alvetra and Werfft AG, UFA-Med, Sursee, Switzerland) on the day of weaning.

GNE: The sows were treated as the sows in GN and received additionally 1 g vitamin E as top dressing on the daily ration

According to Britt *et al.* (4) high ambient temperature affects the hypothalamo-hypophyseal-ovarian axis and diminishes gonadotropin production. Exogenous gonadotropin treatment might acts by overriding this effect (4).

There were no significant differences in weaning to service intervals and regular returns to service between the sows from Groups 1 and 2. Sows from Group 3 had longer ($P < 0.001$) weaning to service intervals and more ($P < 0.001$) regular returns to service during hot weather periods compared with the sows from Groups 1 and 2. Irregular returns to service were low and showed no significant differences between the groups. Farrowing rates and litter sizes were significantly ($P < 0.01$) better in Groups 1 and 2 compared with the Group 3.

The European wild pig (*Sus scrofus*) has strong seasonal reproductive patterns (8). Mating activity peaks in late autumn – early winter and is followed by birth of a litter in spring. Although the litters are weaned in summer, mating ceases between June and September (8). It seems likely that the modern domestic pig has retained such sensitivity to season.

Seasonal infertility in domestic pigs has been recognized for many years in a number of countries including Australia (7), USA (4), UK (8), South Africa (3), and Eastern Europe (Almond and Bilkei, 2005). The severity might vary between countries, but in general the hotter countries experience more severe effects (7, 8).

The mechanisms controlling seasonal breeding activity have been well described for sheep and are determined by changing photoperiod, with decreasing daylength in the early autumn leading to the onset of breeding activity, through the effects of changing photoperiod on gonadotropin secretion.

The mechanisms controlling seasonal breeding activity in the sow are related to gonadotropin production and according to the latest literature can be controlled satisfactorily by parenteral administration of gonadotropins (1, 4, 7, 8).

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EFFECT OF WHEAT PROTEIN – SEITAN, COLOURED BY MICROBIAL NATURAL PIGMENT OF *Monascus purpureus* ON THE ORGANOLEPTIC CHARACTERS OF POULTRY MEAT PRODUCTS

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ABSTRACT

The present times of scientific development include new trends in the manufacture and processing of food. In an effort to offer a new healthy product to consumer, we used wheat protein – seitan, for the production of mosaic poultry salami. Seitan, coloured by microbial natural pigment of *Monascus purpureus* is rich in proteins, does not contain fat and cholesterol and can replace part of the meat of products. Microbial natural pigment of *Monascus purpureus* (type Moguntia, International AG, Europa, the absence of Citrinin was controlled by HPLC analysis) as a powder of Red Yeast Rice was applied to colour – seitan. The average values of tested organoleptic parameters of the manufactured poultry products two days after production resulted in a positive influence ($P < 0.05$) when a part of the meat was replaced by seitan, coloured by Red Yeast Rice. The seitan, coloured by Red Yeast Rice fitted well into the whole and positively affected the general look of the product, also provided a special taste and improved the consistency of the product.

Key words: *Monascus purpureus*; poultry salami; Red Yeast Rice; seitan; wheat proteins

INTRODUCTION

Over the past several years of scientific development included new trends in the manufacture and processing of food. In freezer or refrigerated section of any grocery store we can find products that can be substituted for meat (11, 12). Meat analogs tend to be high in protein, and they have varying amount of fat. These products offer entree alternatives for consumers reducing meat in their diets for health reasons, such as concern about developing heart disease, cancer or high blood pressure. From a health perspective, most meat alternatives are cholesterol-free, contain higher amounts of fiber and usually contain less fat than their animal product counterparts (2, 3).

Proteins derived from plant sources are becoming one of the food industry fasted-growing and most-innovative ingredient segments. Through technological advances, many plan protein characteristics have been altered to extend or even imitate meat products (2, 5).

Historically, wheat protein has been used for thousands of years as a meat substitute called seitan in China, Japan, Korea, Russia and the Middle East. Wheat gluten is becoming a very large player in the industry, with new production facilities for gluten and textured wheat products expanding all over the world (5). Increasingly popular in meat systems, wheat proteins offer functional benefits in products such as comminuted/emulsified and coarsely ground meats. Vegetable protein products also help improve the taste, texture and quality of the final product (14).

Colour is a very important attribute of meats and meat substitute wheat protein and is subject to great changes during processing. Therefore, the addition of artificial dyes or natural pigments is often required to make meat products attractive to the consumer. However, artificial colouring of sausages is not usually permitted. In keeping with the world trend of manufacture all natural foods, i. e. without any synthetic additives is typical to apply Red Yeast Rice to improve the colour and probably their preservation and flavour too (1, 14).

Red Yeast Rice is a unique dietary supplement derived from *Monascus purpureus* Went Yeast, a traditional Chinese medicine that contains modest levels of naturally occurring HMG-CoA reductase inhibitors. With diet and exercise, Red Yeast Rice helps promote healthy cholesterol levels, naturally. The safety and efficacy of this exclusive, all natural ingredient in helping maintain healthy cholesterol levels have been documented in numerous international clinical studies (9, 13, 17).

Aside from the colouring effects, adding Red Yeast Rice to meat products has preserving affects better than the usual nitrite (12). Due the use of this microbial colorant we can achieve a red colour in meat products requested by consumers and also conservation of the products by reducing the activity of the toxins produced by microorganisms (18).

The aim of our study was to prepare an innovatory poultry meat product in which part of the meat was replaced by seitan, coloured by Red Yeast Rice.

MATERIAL AND METHODS

To produce a new poultry product we used a meat preparation produced in Hydina ZK Košice and coloured wheat protein – seitan to replace a part of the meat preparation in the products.

Seitan was made from wheat flour that was mixed with water and needed. The dough was repeatedly rinsed and mixed to remove the starch and some bran until a gluten was obtained. The dough was then boiled in water.

Microbial natural pigment *Monascus purpureus* (type Moguntia, International AG, Europa, the absence of Citrinin was controlled by HPLC analysis) as a powder of Red Yeast Rice was applied to colour – seitan (two grams of red fermented rice per 1 000 grams of seitan) before boiling the dough.

We prepared three products. The first product (M) was mosaic poultry salami with the seitan addition of 300 g .1 000 g^{-1} the poultry preparation. Seitan was modified to fine mosaic with grain 0.3–0.5 cm. In the second product (H) of mosaic poultry salami the seitan was cut in 0.8–1.0 cm^3 and mixed with the poultry preparation in the amount of 150 g .1 000 g^{-1} the poultry preparation. The third product (C) of poultry salami was a control sample without the seitan addition.

The products were placed into a polyamide cover (Ø 45 mm) and heat treated for 10 min at 70 °C. The samples of the products were stored at 4 °C for 2 and 7 days and at –18 °C for 30 days.

All products were organoleptic evaluated and microbiologically examined on 2, 7, and 30 days after production.

Six professionals evaluated organoleptic characteristics of the manufactured products. They worked according methodic instructions for organoleptic evaluation on five-point scale for color, taste, consistency, cutting view and inner view. Emphasis during the evaluation of the quality of the products was put on the characteristics, which are most important for consumers – taste and consistency. Each kind of the products was evaluated anonymously, without packaging and each with its own number, so that the evaluation was objective. The results were evaluated by the statistic Friedman test and Kruskal – Wallis test.

RESULTS

The organoleptic evaluation was done on a five – point scale by a six – member commission evaluating the color, taste, consistency, cutting view and inner view on Days 2, 7 and 30 after production and storage.

The total values of the organoleptic characteristics of the poultry products two and thirty days after production are shown on the Fig. 1.

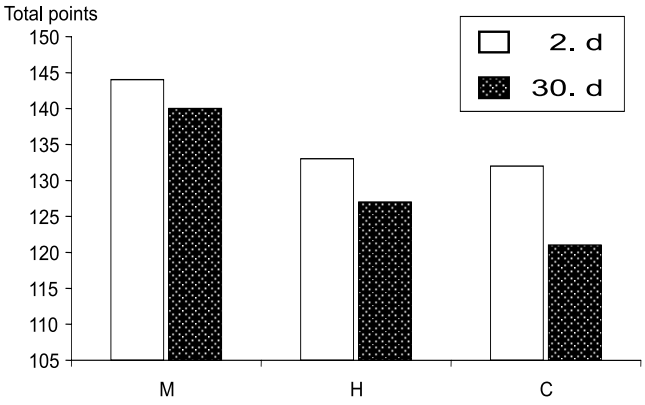

Fig. 1. The total values of the organoleptic characteristics of the poultry products 2 and 30 days after production. M – product with 150 g seitan addition, H – product with 300 g seitan addition, C – control

Significant difference ($P < 0.05$) was noted between the innovated poultry product (M) – mosaic poultry salami with the seitan addition to the amount of 300 g .1 000 g^{-1} in the poultry preparation and the poultry product (C) used as a control on the second and also the thirtieth day after manufacture.

The average values of tested organoleptic parameters of the manufactured poultry products two days after production resulted in a positive influence ($P < 0.05$) when a part of the meat was replaced by seitan, coloured by Red Yeast Rice (Tab. 1).

Table 1. The average values of tested organoleptic parameters of the manufactured poultry products 2 and 30 days after production

Organoleptic parameters	M		H		C	
	2days	30 days	2days	30 days	2days	30 days
Colour	4.83	4.83	4.66	4.66	4.16	4.00
Taste	4.83	4.83	4.66	4.66	4.83	4.50
Consistency	5.00	4.66	4.50	4.00	4.66	3.83
Cutting view	4.83	4.83	4.16	3.83	4.33	3.83
Inner view	4.50	4.16	4.16	4.00	4.00	4.00

M – product with 300 g seitan addition
H – product with 150 g seitan addition
C – control

The product M gained the best results. The seitan, coloured by Red Yeast Rice fitted well into the whole and positively affected the general look of the product. Furthermore, this coloured seitan also provided a special taste and improved the consistency of the product.

The organoleptic evaluation after seven days of storage at cooled temperature was similar to results from the second day after production. After the thirty days storage in a refrigerator was determined the stability and the influence of the seitan on the organoleptic characteristics (Tab. 1).

The results of the microbiological examination correspond with the values prescribed in the *Slovak Codex Alimentarius.* The red fermented rice addition in our meat products did not confirm the microbiological stability improvement definitely. All manufactured products were negative for the presence of coliforms, *Salmonella* spp and *Staphylococcus* spp.

DISCUSSION

The selection of ingredients is the basic for the production of poultry sausages and salami of uniform standard quality. Although poultry meat is the main material in meat preparation, a number of nonmeat ingredients are included in sausage or salami formulations. These ingredients are commonly known as binders, fillers and extenders. Functional properties of these proteins contribute to structural, emulsifying, binding and gelling qualities in sausages as well as to colour and flavour (16). Vital wheat gluten (80 percent protein) is one of the most important protein products used by meat processors for their functional attributes rather than for their nutritional fortification.

In our study had been prepared the innovatory poultry meat products in which part of the meat was replaced by seitan coloured by Red Yeast Rice.

Historically, *Monascus purpureus* was used in Asian cuisine. In south China, Red yeast Rice has been used as a foodstuff and medicinally (6, 17). In Europe, *Monascus* got popularity by descriptions of Dutch scientists (8, 13). Red Yeast rice has been added to meat products to improve the colour and probably their preservation and flavour too. Their results confirmed that food, prepared with *Monascus*, stimulates the blood circulation, prevents gastric and intestinal complaints, stimulates the digestion, etc. When using natural pigment *Monascus purpureus* food products gain a more intense and stable red colour and improved organoleptic characteristics (2, 3, 4). Moreover application of the natural pigment supposes consumers health protection by decreasing of the intake of salt and manufacture all natural food that is food full natural, without any synthetic additives (5, 7, 8).

Our experiments with the innovatory poultry meat products in which part of the meat was replaced by seitan, coloured by Red Yeast Rice alike the experiments with application of the natural ingredient for the manufacture soya beans, cheeses and meat products (1, 3, 4, 14) show that the certain concentrations of this natural pigment may positively effect the organoleptic characteristics and improve their quality. Significant difference ($P < 0.05$) was noted between the innovated poultry product (M) – mosaic poultry salami with the seitan addition to the amount of 300 g .1 000 g^{-1} in the poultry preparation and the poultry product (C) used as a control on the second and also the thirtieth day after manufacture. The average values of tested organoleptic parameters of the manufactured poultry products two days after production resulted in a positive influence ($P < 0.05$) when a part of the meat was replaced by seitan, coloured by Red Yeast Rice. The product M gained the best results. The seitan, coloured by Red Yeast Rice fitted well into the whole and positively affected the general look of the product. Furthermore, this coloured seitan also provided a special taste and improved the consistency of the product.

Red Yeast Rice is one traditional Chinese material that has been shown in animal and pilot human studies to effectively lower serum lipid levels (10, 15). In conclusion, the authors believe that Red Yeast Rice is a "highly effective and well tolerated dietary supplement that can be used to regulate elevated serum cholesterol and triglycerides" (5). Seitan, as a protein meat alternative, offer functional benefits of increased moisture retention and fat binding, as well as improved fat emulsifying properties (16). Another benefit is the overall appearance and yield enhancement of finished products, such as patties, sausages, loaves, meatballs, hot dogs and luncheon meats.

Application some new technology in food industry requires untraditional processing of foodstuffs with the aim to improve their quality, durability, storage, nutritious value and visual attraction. Replacing a part of the meat in poultry meat products by seitan, coloured by Red Yeast Rice supposes not only fulfillment all of these requirements, but set the positive health aspects from and with seitan and *Monascus* prepared food as a goal.

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