Number 1
2019
Volume 52
52 (1) 1–46
ISSN 1337-9984 (Print)
ISSN 1338-0095 (Online)

Slovak Journal of Animal Science

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

SLOVAK JOURNAL OF ANIMAL SCIENCE

Formerly
Journal of Farm Animal Science

AIMS AND SCOPE

Slovak Journal of Animal Science (ISSN 1337-9984, E-ISSN 1338-0095) is an international peer-reviewed scientific journal published by the National Agricultural and Food Centre – Research Institute for Animal Production Nitra.

SJAS publishes original scientific papers, reviews and short communications on the topics of biotechnology, husbandry and nutrition of livestock animals, quality of animal products as well as animal environment and behaviour.

Slovak Journal of Animal Science (SJAS) is published quarterly.

Online version of the journal (E-ISSN 1338-0095) is available at http://www.sjas.ojs.sk

Annual subscription rates (print journal): 65 € collecting in the editorial office directly,
75 € Slovak Republic, 85 € Czech Republic, € Europe, 105 € ROW.

Slovak Journal of Animal Science is published under the authorization and direction of the National Agricultural and Food Centre - Research Institute for Animal Production Nitra, Slovak Republic.

Editorial office, orders, subscription and distribution: National Agricultural and Food Centre – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic
IČO: 42337402, Phone: +421 376 546 249, e-mail: editor@vuzv.sk
http://www.sjas.ojs.sk, http://www.sjas.sk

Filed at the Ministry of Culture of the Slovak Republic: EV 3659/09.

Slovak Journal of Animal Science, 52, 2019 (1)

Contents

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 52, 2019 (1): 1–8

ISSN 1337-9984
E-ISSN 1338-0095

EFFECT OF L-GLYCINE AND L-CARNITINE ON POST-THAW SEMEN PARAMETERS AND FERTILITY IN CHICKEN

Konda PRANAY KUMAR[1], Bommu SWATHI[1], Murugesan SHANMUGAM[2]*

[1]Department of Physiology,College of Veterinary Science, Rajendranagar, Hyderabad, India
[2]ICAR-Directorate of Poultry Science, Rajendranagar, Hyderabad, India

ABSTRACT

The effect of supplementing L-glycine and L-carnitine in cryopreservation mixture on post-thaw semen and fertility parameters was studied. Semen from adult PD6 line roosters was collected, pooled and used. The samples after equilibration at 5°C for 30 minutes were mixed with the diluent with the final concentration of supplements L-glycine of 5 or 15 mM and L-carnitine of 1mM along with a cryoprotectant (4 % dimethylsulfoxide; DMSO), and the semen was cryopreserved in 0.5 ml French straws. Different *in vitro* semen quality parameters, fertility and hatchability were assessed in post-thaw samples. Post-thaw sperm motility, live sperm, MTT dye reduction test and seminal plasma lipid peroxidation were significantly lower ($P < 0.05$) in the cryoprotectant only (4 % DMSO) as well as in the L-glycine- and L-carnitine- supplemented treatments. Fertility was significantly ($P < 0.05$) lower the L-glycine- and L-carnitine-supplemented treatments compared to fresh semen, however, hatchability on fertile egg set was similar in all the treatments. The post-thaw semen parameters, fertility and hatchability in L-glycine- and L-carnitine- supplemented treatments were similar to that of the cryoprotectant only treatment. In conclusion, L-glycine and L-carnitine supplementation did not improve post-thaw semen parameters or fertility and hatchability. Thus, inclusion of these compounds in the chicken semen cryopreservation mixture may not provide advantage during the cryopreservation process.

Key words: carnitine; chicken; cryopreservation; fertility; glycine; semen

INTRODUCTION

The sperm survival and fertilizing ability are reduced during semen cryopreservation process due to the effect of freeze-thawing steps on membrane integrity and other functional parameters (Holt, 2000). In the cryopreservation process, high levels of reactive oxygen species (ROS) are formed (Chatterjee and Gagnon, 2001). The avian sperm membrane has higher polyunsaturated fatty acid concentration in comparison to mammalian sperm (Cerolini *et al*., 1997) and is, therefore, highly susceptible to the deleterious effects of lipid peroxidation (LPO). There may be a compromise in the antioxidant system of semen during the cryopreservation process, which further increases the intensity of LPO and potentiates the damaging effects (Li *et al*., 2010; Partyka *et al*., 2012).

L-Carnitine is a quaternary ammonium compound (Bieber, 1988) and water-soluble amino acid naturally biosynthesized in the kidney and liver of animal body from lysine and methionine (Bremer, 1983). It plays a key role in reducing the availability of lipids for peroxidation by facilitating transport of fatty acids into mitochondria for β-oxidation to generate ATP energy (Hinton *et al*.,

***Correspondence:** E-mail: dr_shan@rediffmail.com, shanmugam.murugesan@icar.gov.in
Dr. Shanmugam M., Senior Scientist, ICAR-Directorate of Poultry Science, Rajendranagar, Hyderabad, India-500030
Tel.: : +91-40-24015651
Fax: +91-04-24017002

Received: July 12, 2018
Accepted: August 15, 2018

1979; Neuman *et al*., 2002; Zhai *et al*., 2007). Antioxidant characteristics and anti-apoptotic activities of L-carnitine have a protective role against damaging effects of ROS and may stabilize mitochondrial membrane and DNA structure (Qi *et al*., 2006). L-Carnitine also increases the activity and levels of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase (Neuman *et al*., 2002). Supplementation of L-carnitine in sperm freezing medium prior to freezing has been found to increase post-thaw motility (Banihani *et al*., 2014), acrosomal integrity (Aliabadi *et al*., 2018) and morphology (Bucak *et al*., 2010) of spermatozoa. Supplementation of L-carnitine in extender has resulted in significant improvement in post-thaw sperm motility in rainbow trout (Kutluyer *et al*., 2014) and bull (Sarıözkan *et al*., 2014).

Supplementation of Beltsville extender with 1 and 2 mM L-carnitine significantly improved post-thaw rooster sperm quality parameters resulting in higher total motility, progressive motility, membrane functionality, viability and lower lipid peroxidation (Fattah *et al*., 2017). In another study, 1 mM L-carnitine supplementation in rooster semen cryopreservation mixture improved post-thaw live sperm ratio without apoptosis and membrane reorganization, live sperm ratio without lipid peroxidation and mitochondrial membrane potential, and decreased sperm proportion with detectable DNA fragmentation (Partyka *et al*., 2017). The effect of L-carnitine supplementation in a freezing mixture on fertility in chicken is not known.

Free amino acids in seminal plasma were found to be reduced in infertile patients in comparison to fertile persons, and it was suggested that these amino acids play a protective role for sperm in the hostile vaginal environment (Silvestroni *et al*., 1979). Among the free amino acids, glycine occurs at the second highest concentration in bull seminal plasma (Assumpção *et al*., 2005). Glycine is the smallest and simplest amino acid with only single hydrogen forming its side chain. Amino acids such as glycine, glutamine, histidine and proline have been employed during sperm cryopreservation in different species, such as ram (Khalili *et al*., 2010), stallion (Trimeche *et al*., 1999), goat (Kundu *et al*., 2001) as well as human (Renard *et al*., 1996). Combining amino acids with glycerol or DMSO have shown to improve post-thaw sperm motility in goat (Kundu *et al*., 2001).

To the best of our knowledge, there is no report about the inclusion of L-glycine during chicken semen cryopreservation on post-thaw sperm parameters, fertility and hatchability. Furthermore, the effect of adding L-carnitine during chicken semen cryopreservation on fertility is not known. Against this background, the present study was carried out to reveal the effect of inclusion of L-glycine and L-carnitine in cryopreservation mixture on post-thaw semen parameters, fertility and hatchability in chicken. The information from this study will help in better understanding of the effects of these compounds and possible inclusion during chicken semen cryopreservation.

MATERIAL AND METHODS

Experimental birds and husbandry

The experiment was carried out at the experimental poultry farm of ICAR- Directorate of Poultry Research, Hyderabad, India. PD6 line males derived from multicolored broiler population, which has been selected for shank length for six generations, were used in the experiments. This line is used as male parent line for production of a countrywide popular rural poultry variety *Gramapriya*. The birds were housed in individual cages in an open-sided house. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC/DPR/17/2).

Semen collection and processing for cryopreservation and quality assays

Adult PD6 roosters were trained to respond to abdominal massage technique (Burrows and Quinn, 1937) for the collection of semen. Semen was collected, pooled and kept on ice throughout the experiment. Collected semen was immediately brought to the laboratory over ice in a covered thermocol box, evaluated and processed for cryopreservation. In the laboratory, a portion of the semen was diluted four times in a semen diluent (Table 1) and was used for evaluation of semen quality parameters.

Fifteen PD6 line male birds aged 38 weeks were used for collecting semen and cryopreservation.

Table 1. Composition of semen diluent (Sasaki *et al*., 2010)

Components	g/100 ml
D (+)-glucose	0.2
D (+)-trehalose dehydrate	3.8
L-glutamic acid, monosodium salt	1.2
Potassium acetate	0.3
Magnesium acetate tetrahydrate	0.08
Potassium citrate monohydrate	0.05
BES	0.4
Bis-Tris	0.4
Distilled water	Up to 100 ml
pH	6.8

The pooled semen samples were initially evaluated for sperm concentration. The samples were diluted with a cryoprotectant-free diluent so that the sperm concentration was 4000 x 10^6/ml. The samples were equilibrated at 5 °C for 30 min and were diluted in 1:1 proportion with the diluent containing 8 % dimethyl sulfoxide (DMSO) so that the final concentration of DMSO was 4 % and the final sperm concentration was 2000 x 10^6/ml in each treatment. The effect of supplementing L-glycine (Invitrogen, USA, Cat. No. 15527-013) at 5 and 15 mM concentrations and L-carnitine at 1 mM concentration (SRL Pvt. Ltd., India, Cat. No. 0348283) along with DMSO on cryopreserved semen were studied. During the preliminary trials in the laboratory it was observed that L-glycine concentrations above 15 mM adversely affected the *in vitro* sperm motility and viability. The L-carnitine concentration was selected based on information from previous reports. The semen mixed with test compounds of different treatments was immediately loaded into 0.5 ml French straws and sealed with polyvinyl chloride powder. The filled straws were placed 4.5 cm above the level of liquid nitrogen (LN_2) on a styrofoam raft floating on LN_2 in a thermocol box and exposed to nitrogen vapours for 30 minutes, then the straws were plunged into LN_2, transferred into canisters and stored at -196 °C. Semen straws were stored for a minimum of ten days before further evaluation. Cryopreserved semen after thawing at 5 °C for 100 sec in ice water (Sasaki *et al*., 2010) was evaluated on ten different occasions for progressive sperm motility, live and abnormal sperm, MTT dye reduction test and seminal plasma lipid peroxidation.

Sperm motility and concentration

Sperm motility was recorded as a percentage of progressively motile sperm by placing a drop of diluted semen on a Makler chamber and examining under 200 x magnification. The percentage of sperm with normal, vigorous and forward linear motion was subjectively assessed and scored. The sperm concentration was estimated by measuring optical density using colorimeter (Taneja and Gowe, 1961).

Live and abnormal sperm

Percentages of live and abnormal sperm were estimated by differential staining technique using Eosin-Nigrosin stain (Campbell *et al*., 1953). Semen smear was prepared by mixing one drop of semen with two drops of Eosin-Nigrosin stain and then air dried. Slides were evaluated under high magnification (1000 x). All completely or partially pink-stained sperm were considered as dead and unstained sperm as live. The percentage of live sperm was determined by counting at least 200 sperm cells. The same slides were used for estimating the abnormal sperm percentage that was showing different morphological abnormalities evaluated at 1000 x magnification.

MTT dye reduction test

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction test was carried out in duplicate samples as described by Hazary *et al*. (2001). Briefly, in a tube, 900 µl Nacl–TES, 100 µl of 100 mM glucose, 30 µl semen sample (2 million sperm/µl) and 50 µl of 4 mM MTT dye were added, mixed and incubated for one hour in a shaking water bath at 37 °C. After incubation, 200 µl of 10 % sodium dodecyl sulfate (SDS) in 0.01 M hydrochloric acid (HCl) solution was added to each tube, mixed well and allowed to stand for one hour. The optical density of each sample was measured against blank at 570 nm in a colorimeter (CL 157, Elico Ltd, Hyderabad, India) and the dye reduction activity of sperm was calculated.

Seminal plasma lipid peroxidation

Lipid peroxidation in seminal plasma was measured by thiobarbituric acid method (Hsieh *et al*., 2006). Semen samples were centrifuged at 7000 rpm

for 5 min to separate seminal plasma. To each test tube, 0.9 ml of distilled water and 0.1 ml of seminal plasma were added followed by addition of 0.5 ml of thiobarbituric acid reagent. The tubes were incubated for one hour in a boiling water bath. After cooling the tube contents, absorbance was measured against blank at 534 nm using UV double beam spectrophotometer (Metstar MUV-61PCS, India).

Fertility trials

Fertility trial was conducted by inseminating PD-3 line hens four times at two days interval. Fifty-five PD-3 line females aged 44 weeks were divided equally into five groups with 11 hens per group. The semen straws were thawed at 5 °C for 100 sec in an ice water (Sasaki *et al*., 2010) and inseminated in hens per vagina with a sperm concentration of 200 million/0.1 ml. The control group was inseminated with freshly collected semen (200 million sperm/0.1 ml). Eggs were collected from the second day of the first insemination and stored at 15 °C until incubation. The eggs were incubated at standard conditions in automatic setter/hatcher incubator (VJ Equipments, Pune, India). The eggs were candled on the 18th day of incubation for embryonic development and fertile eggs were transferred into setter compartment. Infertile eggs were broken open to confirm the absence of embryonic development. The chicks hatched on the 21st day of incubation were counted for calculating hatchability.

Statistical analysis

Data were analyzed using SAS 9.2 software and $P < 0.05$ level was considered as significant. Statistical analyses of semen parameters, fertility and hatchability were performed by one-way ANOVA with Tukey's post hoc test. Data having percentage values were arcsine-transformed and analyzed.

RESULTS

The cryopreserved semen samples had significantly ($P < 0.05$) lower post-thaw sperm motility, live sperm and MTT dye reduction activity compared to control (Table 2). The post-thaw abnormal sperm percentage was significantly higher ($P < 0.05$) in cryopreserved semen. However, there was no difference in seminal plasma lipid peroxidation and fertility in comparison to control.

L-carnitine supplementation in the cryopreservation mixture significantly ($P < 0.05$) lowered post-thaw sperm motility, live sperm, MTT dye reduction activity and fertility.

Table 2. Effect of L-Glycine and L-Carnitine on post-thaw semen parameters, fertility and hatchability of cryopreserved chicken semen

Parameters	Control (fresh semen)	4 % DMSO	4 % DMSO + L-Carnitine 1mM	4 % DMSO + L-Glycine 5mM	4 % DMSO + L-Glycine 15mM
Progressive sperm motility (%)	78.0 ± 0.8^{a}	21.5 ± 0.8^{bc}	24.0 ± 1.3^{b}	21.5 ± 0.8^{bc}	19.0 ± 1.0^{c}
Live sperm (%)	89.75 ± 1.45^{a}	24.00 ± 0.54^{bc}	27.55 ± 1.07^{b}	24.90 ± 0.78^{bc}	22.15 ± 1.01^{c}
Abnormal sperm (%)	1.5 ± 0.18^{b}	2.15 ± 0.18^{a}	2.05 ± 0.11a^{b}	2.25 ± 0.11^{a}	2.35 ± 0.18^{a}
MTT dye reduction test (nM of MTT Formazan/min/million sperm)	95.14 ± 2.31^{a}	39.3 ± 1.55^{b}	40.7 ± 1.9^{b}	39.61 ± 1.46^{b}	40.26 ± 0.93^{b}
Seminal plasma lipid peroxidation (nM MDA/ml)	1.5 ± 0.06	1.37 ± 0.03	1.35 ± 0.04	1.39 ± 0.04	1.41 ± 0.03
Fertility (%)	96.05 ± 2.06^{a}	78.14 ± 5.46^{ab}	59.34 ± 7.68^{b}	54.73 ± 10.04^{b}	51.45 ± 7.43^{b}
Hatchability on FES (%)	77.03 ± 3.98	78.65 ± 3.98	84.32 ± 8.87	69.54 ± 8.77	76.10 ± 8.91
No. of eggs incubated	87	86	85	84	82

Values are mean ± SE.
^{a,b,c} Figures bearing different superscripts in a row differ significantly ($P < 0.05$).
DMSO - Dimethylsulfoxide
FES - Fertile Egg Set
MDA - Malondialdehyde

L-glycine supplementation significant ($P < 0.05$) reduced post-thaw sperm motility, live sperm and MTT dye reduction activity. The reduction in motility and live sperm were progressive with increasing L-glycine concentration. The abnormal sperm percentage was significantly higher ($P < 0.05$) in both the concentrations of L-glycine tested. There was no difference in post-thaw seminal plasma lipid peroxidation between L-glycine treatments and control. The fertility from L-glycine supplemented treatments was significantly ($P < 0.05$) lower compared to the control.

Post-thaw semen parameters and fertility of the L-gycine and L-carnitine treatments were not significantly ($P > 0.05$) different in comparison with the cryoprotectant only (4 % DMSO) treatment. No difference in hatchability on fertile egg set (FES) was observed between the treatments.

DISCUSSION

L-carnitine is concentrated at high levels in the seminal plasma and there exists a correlation between total seminal carnitine concentration and sperm motility (Agarwal and Said, 2004). Dietary supplementation of L-carnitine in roosters has been shown to improve sperm concentration and reduce lipid peroxidation (Neuman *et al*., 2002; Zhai *et al*., 2007) and this may be due to the antioxidant and anti-apoptotic action of L-carnitine (Qi *et al*., 2006). Based on the available information it was hypothesized that adding L-carnitine into the cryopreservation mixture may help in improving the post-thaw semen parameters. In the present study addition of L-carnitine into the cryodiluent mixture did not improve post-thaw semen parameters or fertility. This result is in contrast to improvement in post-thaw sperm motility, membrane functionality, sperm viability and decreased lipid peroxidation by the inclusion of L-carnitine at 1 and 2 mM concentrations (Fattah *et al*., 2017). However, at higher concentrations of 4 and 8 mM the total sperm motility was reduced and sperm lipid peroxidation was increased (Fattah *et al*., 2017). Another chicken semen cryopreservation study (Partyka *et al*., 2017) has indicated that L-carnitine at 1 or 5 mM improved post-thaw live sperm percentage without lipid peroxidation, high mitochondrial potential and reduced sperm ratio with DNA fragmentation. In both the earlier studies effect of L-carnitine on fertility from cryopreserved semen was not documented. In human semen cryopreservation, inclusion of L-carnitine improved post-thaw sperm motility and viability but had no effect on sperm DNA oxidation (Banihani *et al*., 2014). In contrast, Duru *et al*. (2000) had reported no effect of acetyl L-carnitine supplementation on post-thaw human sperm motility or membrane integrity. Semen extender supplemented with L-carnitine (2 mM) had no effect on post-thaw sperm motility and pregnancy rate in bovine (Sarıözkan *et al*., 2014). Thus, the beneficial effects of L-carnitine supplementation during semen cryopreservation were not observed across the species or vary with the concentration in chicken. L-carnitine plays important role in β-oxidation of long-chain free fatty acids in mitochondria, wherein L-carnitine transports free fatty acids and derivative of acyl-CoA into mitochondria. The production of ATP and supply of energy for movement of sperm is modulated by L-carnitine. In the present experiment MTT dye reduction test indicating mitochondrial activity was not altered by supplementation of L-carnitine. Furthermore, seminal plasma lipid peroxidation was also not reduced in the L-carnitine supplemented group. The differing results from other reports on L-carnitine in chicken may be due to a difference in breed, diluent or cryopreservation protocols employed (Abouelezz *et al*., 2015; Long, 2006).

In the present study L-glycine in the cryodiluent mixture did not provide any additional advantages in terms of post-thaw semen parameters or fertility. There is no published literature on the use of L-glycine in chicken semen cryopreservation process for comparison. In the striped bass fish sperm L-glycine at all concentrations tested (25 -100 mM) was found to increase the post-thaw sperm motility (He and Woods, 2003). Glycine has also been used as a component in the red jungle fowl semen extender (RFE) and a fertility of 57 % was reported (Rakha *et al*., 2016). The 5 mM concentration of L-glycine tested in the present study was similar to the concentration present in the RFE diluent, however, this as well as higher concentration in the present study resulted in a deleterious effect on post-thaw sperm motility and live sperm. Earlier reports have indicated that the cryoprotective action by amino acids was manifested at lower

concentrations (Kundu *et al*., 2001; Khalili *et al*., 2010). In the biosynthetic process of glutathione in the cell glycine as well as glutamate is involved (Wu *et al*., 2009) and Rakha *et al*. (2016) had suggested that the presence of higher level of glycine might have supported the Indian red jungle fowl sperm against lipid peroxidation of sperm plasma membrane during cryopreservation process. In the present study, there was no difference in seminal plasma lipid peroxidation in treatments having glycine, when compared with control or 4 % DMSO group. The exact mechanism of cell protective action of glycine is not known. It was suggested that small neutral amino acids, such as glycine, stabilize cell membrane protein tertiary structure through their physicochemical effects; furthermore, their metabolism is not required for producing beneficial effects (Baines *et al*., 1990). Similarly, another study suggested that by interaction with the phosphate groups in the sperm plasma membrane phospholipids amino acids could form a layer on the sperm surface (Anchordoguy *et al*., 1988). Glycine inclusion in striped bass semen cryopreservation had increased the sperm mitochondrial function and ATP content (He and Woods, 2003). The authors have quoted two hypothesis for higher mitochondrial function and ATP content; glycine after crossing sperm plasma membrane provides a positive effect on mitochondria (Flipse, 1956) or glycine binding to its receptors on plasma membrane triggers signal transduction that finally protects mitochondrial function and sperm ATP content.

In the present study, there was no improvement in the sperm mitochondrial activity in the glycine supplemented treatments in contrast to that reported on striped bass. Though glycine receptor on sperm membrane in other species has been reported with a role in acrosome reaction (Melendrez and Meizel, 1995), their presence in rooster sperm is yet to be documented.

CONCLUSION

In conclusion, results of the present study indicated that addition of L-carnitine and L-glycine to cryopreservation mixture did not improve post-thaw semen quality and fertility in chicken. Therefore inclusion of these compounds during chicken semen cryopreservation may not provide any additional advantage during the cryopreservation process.

Conflict of Interest

None of the authors have any conflict of interest to declare.

REFERENCES

Abouelezz, F. M. K., Castaño, C., Toledano-Díaz, A., Esteso, M. C., López-Sebastián, A., Campo, J. L. & Santiago-Moreno, J. 2015. Effect of the interaction between cryoprotectant concentration and cryopreservation method on frozen/thawed chicken sperm variables. *Reproduction in Domestic Animals*, 50(1), 135–141.

Agarwal, A. & Said, T. M. 2004. Carnitines and male infertility. *Reproductive Biomedicine Online*, 8(4), 376–384.

Aliabadi, E., Jahanshahi, S., Talaei-Khozani, T. & Banaei, M. 2018. Comparison and evaluation of capacitation and acrosomal reaction in freeze-thawed human ejaculated spermatozoa treated with L-carnitine and pentoxifylline. *Andrologia*, 50(2). https://doi: 10.1111/and.12845

Anchordoguy, T., Carpenter, J. F., Loomis, S. H. & Crowe, J. H. 1988.Mechanisms of interaction of amino acids with phospholipid bilayers during freezing. *Biochimica et Biophysica Acta*, 946(2), 299–306.

Assumpção, T. I., Torres Júnior, R. A. A., Sousa, M. V. & Ricart, C. A. O. 2005. Correlation between fertility and levels of protein, sugar and free amino acids in seminal plasma of Nelore bulls. *Arquivo Brasileiro de Medicina Veterináriae Zootecnia*, 57(1), 55–61.

Banihani, S., Agarwal, A., Sharma, R. & Bayachou, M. 2014. Cryoprotective effect of L-carnitine on motility, vitality and DNA oxidation of human spermatozoa. *Andrologia*, 46(6), 637–641.

Baines, A. D., Shaikh, N. & Ho, P. 1990. Mechanisms of perfused kidney cytoprotection by alanine and glycine. *American Journal of Physiology*, 259(1 Pt 2), F80–F87.

Bieber, L. L. 1988. Carnitine. *Annual Review of Biochemistry*, 57, 261–283.

Bremer, J. 1983. Carnitine--metabolism and functions. *Physiological Reviews*, 63(4), 1420–1480.

Bucak, M. N., Tuncer, P. B., Sarıözkan, S., Başpınar, N., Taşpınar, M., Çoyan, K., Bilgili, A., Akalın, P. P.,

Büyükleblebici, S., Aydos, S., Ilgaz, S., Sunguroğlu, A., & Oztuna, D. 2010. Effects of antioxidants on post-thawed bovine sperm and oxidative stress parameters: antioxidants protect DNA integrity against cryodamage. *Cryobiology*, 61(3), 248–253.

Burrows, W. H. & Quinn, J. P. 1937. The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science*, 16(1), 19–24.

Campbell, R. G., Hancock, J. L. & Rothschild, L. 1953. Counting live and dead bull spermatozoa. *Journal of Experimental Biology*, 30, 44–49.

Cerolini, S., Kelso, K. A., Noble, R. C., Speake, B. K., Pizzi, F. & Cavalchini, L. G. 1997. Relationship between spermatozoan lipid composition and fertility during aging of chickens. *Biology of Reproduction*, 57(5), 976–980.

Chatterjee, S. & Gagnon, C. 2001. Production of reactive oxygen species by spermatozoa undergoing cooling, freezing, and thawing. *Molecular Reproduction and Development*, 59(4), 451–458.

Duru, N. K., Morshedi, M., Schuffner, A. & Oehninger, S. 2000. Semen treatment with progesterone and/or acetyl-L-carnitine does not improve sperm motility or membrane damage after cryopreservation-thawing. *Fertility and Sterility*, 74(4), 715–720.

Fattah, A., Sharafi, M., Masoudi, R., Shahverdi, A., Esmaeili, V. & Najafi, A. 2017. L-Carnitine in rooster semen cryopreservation: Flow cytometric, biochemical and motion findings for frozen-thawed sperm. *Cryobiology*, 74, 48–153.

Flipse, R. J. 1956. Metabolism of glycine by bovine spermatozoa. *Science*, 124, 228.

Hazary, R. C., Chaudhuri, D. & Wishart, G. J. 2001. Application of an MTT reduction assay for assessing sperm quality and predicting fertilising ability of domestic fowl semen. *British Poultry Science*, 42(1), 115–117.

He, S. & Woods, L. C. 2003. Effect of glycine and alanine on short term storage and cryopreservation of striped bass (*Morone saxatilis*) spermatozoa. *Cryobiology*, 46(1), 17–25.

Hinton, B. T., Snoswell, A. M. & Setchell, B. P. 1979. The concentration of carnitine in the luminal fluid of the testis and epididymis of the rat and some other mammals. *Journal of Reproduction and Fertility*, 56(1), 105–111.

Hsieh, Y. Y., Chang, C. C. & Lin, C. S. 2006. Seminal malondialdehyde concentration but not glutathione peroxidase activity is negatively correlated with seminal concentration and motility. *International Journal of Biological Sciences*, 2(1), 23–29.

Holt, W. V. 2000. Basic aspects of frozen semen storage. *Animal Reproduction Science*, 62(1-3), 3–22.

Khalili, B., Jafaroghli, M., Farshad, A. & Paresh-Khiavi, M. 2010. The effects of different concentrations of glycine and cysteine on the freezability of Moghani ram spermatozoa. *Asian-Australasian Journal of Animal Sciences*, 23(3), 318–325.

Kundu, C. N., Das, K. & Majumder, G. C. 2001. Effect of amino acids on goat cauda epididymal sperm cryopreservation using a chemically defined model system. *Cryobiology*, 42(1), 21–27.

Kutluyer, F., Kayim, M., Öğretmen, F., Büyükleblebici, S. & Tuncer, P. B. 2014. Cryopreservation of rainbow trout *Oncorhynchus mykiss* spermatozoa: effects of extender supplemented with different antioxidants on sperm motility, velocity and fertility. *Cryobiology*, 69(3), 462–466.

Li, Z., Lin, Q., Liu, R., Xiao, W. & Liu, W. 2010. Protective effects of ascorbate and catalase on human spermatozoa during cryopreservation. *Journal of Andrology*, 31(5), 437–444.

Long, J. A. 2006. Avian Semen Cryopreservation: What Are the Biological Challenges? *Poultry Science*, 85(2), 232–236.

Melendrez, C. S. & Meizel, S. 1995. Studies of porcine and human sperm suggesting a role for a sperm glycine receptor/Cl- channel in the zona pellucida-initiated acrosome reaction. *Biology of Reproduction*, 53(3), 676–683.

Neuman, S. L., Lin, T. L. & Hester, P. Y. 2002. The effect of dietary carnitine on semen traits of white leghorn roosters. *Poultry Science*, 81(4), 495-503.

Partyka, A., Łukaszewicz, E. & Niżański, W. 2012. Effect of cryopreservation on sperm parameters, lipid peroxidation and antioxidant enzymes activity in fowl semen. *Theriogenology*, 77(8), 1497–1504.

Partyka, A., Rodak, O., Bajzert, J., Kochan, J. & Niżański, W. 2017. The effect of L-carnitine, hypotaurine, and taurine supplementation on the quality of cryopreserved chicken semen. *BioMedical Research International*, Article ID 7279341. https://doi.org/10.1155/2017/7279341

Qi, S. N., Zhang, Z. F., Wang, Z. Y., Yoshida, A. & Ueda, T. 2006. L-carnitine inhibits apoptotic DNA fragmentation induced by a new spin-labeled derivative of podophyllotoxin via caspase-3 in Raji cells. *Oncology Reports*, 15(1), 119–122.

Rakha, B. A., Ansari, M. S., Akhter, S., Hussain, I. & Blesbois, E. 2016. Cryopreservation of Indian red

jungle fowl (*Gallus gallus murghi*) semen. *Animal Reproduction Science*, 174, 45–55.

Renard, P., Grizard, G., Griveau, J. F., Sion, B., Boucher, D. & Le, L. D. 1996. Improvement of motility and fertilization potential of postthaw human sperm using glutamine. *Cryobiology*, 33(3), 311–319.

Sarıözkan, S., Bucak, M. N., Tuncer, P. B., Büyükleblebici, S. & Cantürk, F. 2014. Influence of various antioxidants added to TCM-199 on post-thaw bovine sperm parameters, DNA integrity and fertilizing ability. *Cryobiology*, 68(1), 129–133.

Sasaki, K., Tatsumi, T., Tsutsui, M., Niinomi, T., Imai, T., Naito, M., Tajima, A. & Nishi, Y. 2010. A method for cryopreserving semen from Yakido roosters using n-methylacetamide as a cryoprotective agent. *The Journal of Poultry Science*, 47(4), 297–301.

Silvestroni, L., Morisi, G., Malandrino, F. & Frajese, G. 1979. Free amino acids in semen: measurement and significance in normal and oligozoospermic men. *Archives of Andrology*, 2(3), 257–261.

Taneja, G. C. & Gowe, R. S. 1961. Spermatozoa concentration in the semen of two breeds of fowl estimated by three different methods. *Poultry Science*, 40(3), 608–615.

Trimeche, A., Yvon, J. M., Vidament, M., Palmer, E. & Magistrini, M. 1999. Effects of glutamine, proline, histidine and betaine on post-thaw motility of stallion spermatozoa. *Theriogenology*, 52(1), 181–191.

Wu, G., Bazer, F. W., Davis, T. A., Kim, S. W., Li, P., Marc Rhoads, J., Carey Satterfield, C., Smith, S. B., Spencer, T. E. & Yin, Y. 2009. Arginine metabolism and nutrition in growth, health and disease. *Amino Acids*, 37(1), 153–168.

Zhai, W., Neuman, S. L., Latour, M. A. & Hester, P. Y. 2007. The effect of L-Carnitine on semen traits of white leghorns. *Poultry Science*, 86(10), 2228–2235.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 52, 2019 (1): 9–15

ISSN 1337-9984
E-ISSN 1338-0095

MINERAL PROFILE ANALYSIS OF OILSEEDS AND THEIR BY-PRODUCTS AS FEEDING SOURCES FOR ANIMAL NUTRITION

Renata KOLLÁTHOVÁ[1]*, Branislav VARGA[1], Eva IVANIŠOVÁ[2], Daniel BÍRO[1], Michal ROLINEC[1], Miroslav JURÁČEK[1], Milan ŠIMKO[1], Branislav GÁLIK[1]

[1]Slovak University of Agriculture in Nitra, Department of Animal Nutrition, Nitra, Slovak Republic
[2]Slovak University of Agriculture in Nitra, Department of Plant Storage and Processing, Nitra, Slovak Republic

ABSTRACT

The aim of this study was to determine the dry matter (DM) and crude ash content, as well as the mineral content of oilseeds and their by-products, cakes. Four samples of oilseeds (sunflower, soybean, flaxseed and rapessed) were analysed as seeds and cakes. Analyzed crops were grown at the University Experimental Farm in Kolíňany. Cakes were obtained by using FARMER 10 pressing unit. Dry matter and crude ash were determined by standard laboratory methods and procedures. Mineral nutrient profile analysis was performed by using the High Resolution Continuum Source Atomic Absorption Spec trometer contrAA 700 for calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn). To analyze phosphorus (P) content, 6400 Spectrophotometer was used. Significant differences ($P < 0.05$) in DM and crude ash content, as well as in mineral composition of analyzed seeds and cakes, were found. The most represented macroelements were K (soybean seed and soybean cake over 20 $g.kg^{-1}$ of DM) and P (rapeseed cake 12.23 $g.kg^{-1}$ of DM and sunflower cake 11.42 $g.kg^{-1}$ of DM). On the other hand, Na was present in the analyzed samples the least, mostly below 1 $g.kg^{-1}$ of DM. From microelements, the highest values were observed for Fe (with the maximum for rapeseed cake 107.94 $mg.kg^{-1}$ of DM and soybean cake 88.97 $mg.kg^{-1}$ of DM) and Zn (with the maximum for sunflower cake 58.94 $mg.kg^{-1}$ of DM and rapeseed cake 52.38 $mg.kg^{-1}$ of DM). Rapeseed and rapeseed cake have significantly ($P < 0.05$) proven to be the richest in Mn content (32.96 $mg.kg^{-1}$ of DM and 54.07 $mg.kg^{-1}$ of DM, respectively), but on the other hand they contained the least amount of Cu (6.82 $mg.kg^{-1}$ of DM and 8.90 $mg.kg^{-1}$ of DM, respectively).

Key words: oilseeds; oliseed cakes; mineral content; macroelemnets; microelements

INTRODUCTION

Oilseeds are considered an important source of many nutrients and minerals in both human and animal nutrition (Das *et al.*, 2017). Despite their high energy value and high protein content, oilseeds in feed industry are used only marginally, because they often contain antinutrients, which negatively affect the quality of animal products or animal health (Zeman *et al.*, 2006). Due to their high fat content they are mainly used for oil production (Gunstone, 2002). In animal nutrition only by-products from the production of vegetable or technical oils, such as extracted meals or cakes, are used with great importance. Ramachandran *et al.* (2007) and Gálik *et al.* (2016) reported that in recent decades there has been an increasing interest in the use of organic by-products of industrial processing as feed ingredients for economic and environmental reasons.

***Correspondence:** E-mail: xkollathova@uniag.sk
Renata Kolláthová, Slovak University of Agriculture in Nitra,
Department of Animal Nutrition, Tr. A. Hlinku 2,
949 76 Nitra, Slovak Republic
Tel.: : +421 37 641 4502

Received: September 20, 2018
Accepted: November 20, 2018

In terms of mineral content oilseeds are considered as deficient, which means that they are nutritional sources of only a small range of minerals. From macroelements they are mostly rich in phosphorus, much like grain cereals. From microelements, oilseeds (including soy) can be a very good source of iron and zinc. Other microelements are typically found only in trace amounts (Gálik *et al.*, 2016). The mineral content of oilseeds is influenced by a number of factors, such as species, soil and climatic conditions, as well as agrotechnics and breeding (Gálik *et al.*, 2016).

MATERIAL AND METHODS

In the experiment, dry matter (DM) and crude ash content, as well as minerals content of oilseeds and their cakes were determined. Four samples of oilseeds (sunflower, soybean, flaxseed and rapeseed) in triplicate were analysed as seeds and cakes. Analyzed crops were grown in University Experimental Farm in Kolíňany under identical agroclimatic conditions. Harvest of seeds was realized at the stage of full maturity. Seeds were processed in the Laboratory of fats and oils (AgroBio Tech Research Centre of the Slovak University of Agriculture in Nitra). FARMER 10 pressing unit (Farmet, Czech Republic) was used. Laboratory samples were analysed in the Laboratory of Quality and Nutritive Value of Feeds at the Department of Animal Nutrition at the Slovak Agricultural University. DM and crude ash were determined by standard laboratory methods and procedures (EC No 152/2009). The contents of mineral nutrients were determined by High Resolution Continuum Source Atomic Absorption Spectrometer contrAA 700 (ANALYTIK JENA) (Ca, Mg, Na, K, Zn, Cu, Fe, Mn) and 6400 Spectrophotometer (P). The determination of individual element content was based on the absorptions measured at the following wavelengths: Ca content was detected at 422.7 nm, P at 666 nm, Mg at 285.2 nm, Na at 589.0 nm, K at 766.5 nm, Zn at 213.9 nm, Cu at 324.7 nm, Fe at 248.3 nm and Mn at 279.5 nm. To calculate basic statistic characteristics, to determine significance of differences and to compare results a one-way ANOVA and t-test were performed at $P < 0.05$ level. The SAS statistical package was used (SAS Inc., New York City, USA).

RESULTS AND DISCUSSION

Dry matter and Crude ash

In analyzed seeds the DM content ranged from 94.32 to 95.88 % (Table 1.), however, differences were significant ($P < 0.05$). The lowest DM content was observed for soybean. Ítavo *et al.* (2015) reported higher value for DM in soybean seed (981.2 $g.kg^{-1}$) in comparision to our data. The highest DM content was observed in rapeseed, though it was lower than reported by Rymer and Short (2003).

The crude ash content of feed materials is often wrongly mistaken for mineral content. In terms of quality, safety and availability of nutrients in nutrition, however, a lower content of crude ash in nutrient sources is desired (Gálik *et al.*, 2016). As shown in Table 1., among seeds, significantly lowest crude ash content ($P < 0.05$) was detected in sunflower seed and rapeseeds. Chung *et al.* (2009) reported higher, whilst Nadeem *et al.* (2010) – lower crude ash content in whole sunflower seeds. In comparision with Ewing *et al.* (1997), Santos *et al.* (2009) and Carré *et al.* (2016) a lower content of crude ash in rapeseed was found. Significantly highest crude ash content ($P < 0.05$) was revealed in soybean seeds. This corresponds to the results

Table 1. Dry matter ($g.kg^{-1}$) and crude ash ($g.kg^{-1}$ of DM) content of analyzed oilseeds

	Sunflower seed	Soybean seed	Flaxseed	Rapeseed
	Mean ± SD (n = 2)			
Dry matter	947.25 ± 0.35^{a}	943.20 ± 0.10^{b}	948.25 ± 0.15^{c}	958.80 ± 0.01^{d}
Crude ash	36.20 ± 0.20^{a}	53.40 ± 1.20^{b}	39.80 ± 0.20^{c}	36.10 ± 0.20^{a}

SD: standard deviation. Values followed by different letters within a row are significant at the level 0.05.

published by Rymer and Short (2003), whilst lower content of crude ash in soybean seed was reported by Ciabotti *et al.* (2016) and higher – by Goes *et al.* (2010). Differences in crude ash content compared to the literature sources may be due to topographic and climatic factors.

Table 2. demonstrates that the DM content of cakes differed only slightly compared to the DM content of seeds. The lowest DM content was determined in rapeseed cake and the highest DM content was observed in soybean cake. Leming and Lember (2005) found lower DM content in rapeseed cake (917 $g.kg^{-1}$). Analysed DM content in soybean cake was higher than reported by Geremew *et al.* (2015). These discrepancies in DM content may in general result from differences in moisture absorption between spieces due to genetic variations, processing and storage of samples. The analyzed differences in DM content of cakes were significant ($P < 0.05$).

Compared to seeds, the crude ash content of cakes was higher with a maximum for rapeseed cake and the minimum for flaxseed cake. According to Geremew *et al.* (2015), lower content of ash in flaxseed cake was found. Significant differences ($P < 0.05$) in the ash content of all the analyzed cakes were observed (Table 2.).

Macroelements

The mineral content of oilseeds is influenced by several factors such as species, soil and climatic conditions, as well as agro-technology and breeding (Gálik *et al*., 2016). The content of macroelements in analyzed seeds is shown in Table 3. In the content of macroelements (Ca, P, Mg, Na and K), significant differences ($P < 0.05$) between the analyzed seeds were observed. Significantly highest Ca content ($P < 0.05$) was detected in rapeseed (3.56 $g.kg^{-1}$ of DM), which corresponds to the results published by McKevith (2005). On the other hand, sunflower seed contained significantly ($P < 0.05$) lowest amount of Ca (2.32 $g.kg^{-1}$ of DM). According to McKevith (2005) in terms of Ca content, sesame seeds (an average of 7 $g.kg^{-1}$ of DM), rapeseeds (4 $g.kg^{-1}$ of DM) and flaxseeds (2 $g.kg^{-1}$ of DM) can be considered as the most qualitative. On the contrary, groundnut and soybean seeds are deficient in calcium content. In the case of P content, we found quantitatively higher values compared to Ca. Significantly highest ($P < 0.05$) amount of P was detected in flaxseed (8.55 $g.kg^{-1}$ of DM) and the lowest in rapeseed (5.53 $g.kg^{-1}$ of DM). Morris (2007) reported lower amount of P in flaxseed (6.22 $g.kg^{-1}$ of DM). From oilseeds, the most P rich are cotton seeds, rapeseeds, sesame and flax seeds (McKevith, 2005). Very similar levels of Ca and P in oilseeds were also reported by Petrikovič *et al*. (2000), as well as Gálik *et al*. (2016). In terms of Ca and P content, oilseeds in diets are considered primarily a nutritional source of P. In Mg content, we observed significant differences ($P < 0.05$) between sunflower, soybean and rapeseeds. Compared to other seeds, the significantly ($P < 0.05$) highest Mg content was found in flaxseed (3.44 $g.kg^{-1}$ of DM). A slightly higher average Mg content for flaxseed was reported by Petrikovič *et. al.* (2000) and Morris (2007). Mg content detected for soybean seed was significantly ($P < 0.05$) lowest. Na is considered as an important cation in animal nutrition, which retains water in the body (Gálik *et al*., 2011). Its content is generally low in oilseeds and must be nutritionally supplied from other sources (Bíro *et al*. 2014). In analyzed seeds the content of Na ranged from 0.17 $g.kg^{-1}$ of DM (sunflower seed) to 0.43 $g.kg^{-1}$ of DM (flaxseed), and these differences were significant ($P < 0.05$). In comparition with Morris (2007), higher amount of Na in flaxseed was found. K is antagonistic to Na and promotes

Table 2. Dry matter ($g.kg^{-1}$) and crude ash ($g.kg^{-1}$ of DM) content of analyzed cakes

	Sunflower cake	Soybean cake	Flaxseed cake	Rapeseed cake
	Mean ± SD (n = 2)			
Dry matter	947.60 ± 0.30[a]	950.35 ± 0.05[b]	946.55 ± 0.25[c]	945.00 ± 0.20[d]
Crude ash	54.10 ± 0.30[a]	56.10 ± 0.10[b]	51.00 ± 0.10[c]	61.90 ± 0.30[d]

SD: standard deviation. Values followed by different letters within a row are significant at the level 0.05.

Table 3. Content of macroelements in analyzed oilseeds (g.kg^{-1} of DM)

	Sunflower seed	Soybean seed	Flaxseed	Rapeseed
	Mean ± SD (n = 2)			
Ca	2.32 ± 0.06^{a}	2.56 ± 0.10^{b}	2.95 ± 0.01^{c}	3.56 ± 0.05^{d}
P	6.91 ± 0.08^{a}	7.03 ± 0.00^{a}	8.55 ± 0.00^{b}	5.53 ± 0.08^{c}
Mg	3.35 ± 0.10^{a}	2.09 ± 0.01^{b}	3.44 ± 0.03^{a}	2.26 ± 0.06^{c}
Na	0.17 ± 0.00^{a}	0.22 ± 0.00^{b}	0.43 ± 0.04^{c}	0.18 ± 0.00^{d}
K	8.69 ± 0.10^{a}	20.23 ± 0.37^{b}	9.52 ± 0.09^{c}	8.00 ± 0.36^{d}

SD: standard deviation. Values followed by different letters within a row are significant at the level 0.05.

the release of water from the body (Gálik *et al*., 2011). The highest content of K was detected in soybean seed (20.23 g.kg^{-1} of DM). According to Van Eys *et al*. (2004) and Aletor *et al*. (2010) higher amount of K in soybean seed was observed. On the contrary, the lowest amount of K was detected in rapeseed (8.0 g.kg^{-1} of DM). Differences in K content of soybean and rapeseed were significant ($P < 0.05$).

The content of macroelements in cakes is shown in Table 4. Between the analyzed cakes, significant differences ($P < 0.05$) in the content of Ca and P were found. In comparison with seeds, quantitatively higher values were observed, with the highest levels of Ca and P detected for rapeseed cake. According to Leming and Lember (2005) lower contents of Ca and P in rapeseed cake were found. The Mg content in cakes ranged from 2.09 g.kg^{-1} of DM (soybean cake) to 4.41 g.kg^{-1} of DM (flaxseed cake). Just as in analyzed seeds, only traces of Na in cakes were found. The highest amount of K was observed in soybean cake (20.47 g.kg^{-1} of DM) and the lowest in flaxseed cake (12.41 g.kg^{-1} of DM).

Microelements

Recently, only a limited number of papers has been published on the content of microelements in oilseeds and by-products derived from their industrial processing. Table 5. lists the content of selected microelements in analyzed seeds. The highest Cu content was found by a laboratory analysis in sunflower seed (21.25 mg.kg^{-1} of DM) and the lowest in rapeseed (6.82 mg.kg^{-1} of DM). These results correspond with the work of Petrikovič *et al*. (2000), although in the case of sunflower seed, the authors report an average Cu content only 10.9 mg.kg^{-1} of DM. Mainly flax and sunflower seeds are characterized by a higher Fe content, typically over 100 mg.kg^{-1} of DM (Petrikovič *et al*., 2000). However, Morris (2007) provides half of this amount of Fe in flaxseeds. In analyzed oilseeds the Fe content was between 38.30 mg.kg^{-1} of DM (sunflower seed) and 72.85 mg.kg^{-1} of DM (rapeseed). Nadeem *et al*. (2010) detected higher Fe content in sunflower seeds (68.61 mg.kg^{-1} of DM). However, in another work McKevith (2005) reported a very similar Fe content in flaxseed,

Table 4. Content of macroelements in analyzed cakes (g.kg^{-1} of DM)

	Sunflower cake	Soybean cake	Flaxseed cake	Rapeseed cake
	Mean ± SD (n = 2)			
Ca	3.28 ± 0.00^{a}	3.49 ± 0.00^{b}	3.61 ± 0.06^{c}	6.63 ± 0.07^{d}
P	11.42 ± 0.16^{a}	6.08 ± 0.00^{b}	9.52 ± 0.32^{c}	12.23 ± 0.00^{d}
Mg	4.22 ± 0.04^{a}	2.09 ± 0.10^{c}	4.41 ± 0.01^{b}	4.08 ± 0.35^{ab}
Na	0.29 ± 0.00^{a}	0.23 ± 0.00^{b}	0.34 ± 0.00^{c}	0.18 ± 0.00^{d}
K	13.82 ± 0.60^{a}	20.47 ± 0.00^{b}	12.41 ± 0.23^{a}	13.81 ± 0.07^{a}

SD: standard deviation. Values followed by different letters within a row are significant at the level 0.05.

Table 5. Content of microelements in analyzed oilseeds (mg.kg^{-1} of DM)

	Sunflower seed	Soybean seed	Flaxseed	Rapeseed
	Mean ± SD (n = 2)			
Cu	21.25^{a} ± 1.23^{a}	13.15 ± 0.15^{b}	14.24 ± 0.00^{c}	6.82 ± 0.00^{d}
Fe	38.30 ± 2.83^{a}	71.83 ± 0.09^{b}	59.53 ± 0.01^{c}	72.85 ± 0.96^{d}
Mn	15.56 ± 0.07^{a}	25.82 ± 0.53^{b}	10.91 ± 0.22^{c}	32.96 ± 0.00^{d}
Zn	47.30 ± 0.20^{a}	48.13 ± 0.44^{b}	45.19 ± 1.41^{a}	35.20 ± 0.07^{c}

SD: standard deviation. Values followed by different letters within a row are significant at the level 0.05.

rapeseed, soybean and sunflower seeds compared to our results. These findings point out that this issue is not covered by a sufficient number of relevant studies and works that would be available. In Mn content also a wide range from 10.91 mg.kg^{-1} of DM (flaxseed) to 32.96 mg.kg^{-1} of DM (rapeseed) was detected. In comparision with the work of Nadeem *et al*. (2010), lower amount of Mn in sunflower seed was observed. The last analyzed microelement in oilseeds was Zn, a microelement, whose importance in nutrition is mainly related to the immunization of animals (Gálik *et al*., 2011). According to Petrikovič *et al*. (2000) a higher content of Zn occurs mainly in flaxseed and rapeseeds. However, according to McKevith (2005), Zn in flaxseed is found in a lower amount, the higher content is typical for sunflower seed and only trace amounts of Zn occur in soybean seed and rapeseed. In the analyzed seeds, Zn was present in a range from 35.20 mg.kg^{-1} of DM (rapeseed) to 48.13 mg.kg^{-1} of DM (soybean seed). Anuonye *et al*. (2010) reported Zn content in soybean seed between 30 and 50 mg.kg^{-1} of DM. With the exception of the Zn content, all differences in the microelements content between the seeds were significant ($P < 0.05$).

Table 6. shows the content of selected microelements in analyzed cakes. Similarly to seeds, in addition to Zn content, significant differences were found ($P < 0.05$). The significantly ($P < 0.05$) highest content of Cu was obtained in sunflower cake, the significantly ($P < 0.05$) highest content of Fe and Mn was detected in rapeseed cake. The highest content of Zn was found in sunflower cake.

CONCLUSION

The aim of this study was to determine DM and crude ash content as well as mineral profile of four oilseeds (sunflower, soybean, flaxseed and rapessed) and their cakes. Significant ($P < 0.05$) differences in the composition of analyzed seeds and cakes, as well as in their mineral content were found. Compared with other studies of various authors

Table 6. Content of microelemnets in analyzed cakes (mg.kg^{-1} of DM)

	Sunflower cake	Soybean cake	Flaxseed cake	Rapeseed cake
	Mean ± SD (n = 2)			
Cu	31.98 ± 0.01^{a}	14.68 ± 0.52^{b}	17.75 ± 0.74^{c}	8.90 ± 0.00^{d}
Fe	58.78 ± 1.47^{a}	88.97 ± 0.68^{b}	78.60 ± 0.03^{c}	107.94 ± 1.46^{d}
Mn	21.05 ± 0.53^{a}	25.62 ± 0.37^{b}	13.73 ± 0.30^{c}	54.07 ± 0.13^{d}
Zn	58.94 ± 0.65^{a}	44.77 ± 0.67^{b}	48.60 ± 0.62^{a}	52.38 ± 0.58^{c}

SD: standard deviation. Values followed by different letters within a row are significant at the level 0.05.

these differences are probably related to the variety and environment in which the crops are grown. The experimental results show that oilseeds and by-products from oil production can be a good source of K and P, Fe and Zn but are deficient in Na and Cu content. The wide range in microelement content in analyzed samples points out that this issue is not covered by a sufficient number of relevant studies and works that would be available. In this way, further research in the future is needed.

ACKNOWLEDGEMENTS

The study was supported by the Grant Agency of the Slovak Ministry of Education, Science, Research and Sport and the Slovak Academy of Sciences (project n. 1/0723/15). This work was co-funded by the European Community project no 26220220180: Building the Research Centre „AgroBioTech“.

REFERENCES

Aletor, O. 2010. Soyabean meal versus soyabean protein isolate: A comparative study of the nutritive and functional attributes. *Journal of Food Agriculture & Environment*, 8(2), 34–38.

Anuonye, J. C., Onuh, J. O., Egwim, E. & Adeyemo, S. O. 2010. Nutrient and Antinutrient Composition of Extruded Acha/Soybean Blends. *Journal of Food Processing and Preservation*, 34, 680–691.

Bíro, D., Juráček, M., Šimko, M., Gálik, B. & Rolinec, M. 2014. *Preserving and processing of feed*. 1st ed., Nitra: Slovak University of Agriculture, p. 223.

Carré, P., Citeau, M., Robin, G. & Estorges, M. 2016. Hull content and chemical composition of whole seeds, hulls and germs in cultivars of rapeseed (*Brassica napus*). *Oilseeds & fats Crops and Lipids*, 23(3), Article Number A302. DOI: 10.1051/ocl/2016013

Ciabotti, S., Silva, A. C. B. B., Juhasz, A. C. P., Mendonça, C. D., Tavano, O. L., Mandarino, J. M. G. & Gonçalves, C. A. A. 2016. Chemical composition, protein profile, and isoflavones content in soybean genotypes with different seed coat colors. *International Food Research Journal*, 23(2), 621–629.

Chung, S., Oliveira, C. R. C., Souza, J. G., Aguiar, E. M. & Brasil, D. F. 2009. Avaliação físico-química da torta de girassol (*Helianthus annuss* L.) para a utilização na alimentação animal. In *Congresso Brasileiro de Zootecnia*. Águas de Lindóia: Faculdade de Zootecnia e Engenharia de Alimentos.

Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (Text with EEA relevance).

Das, P., Laishram, P. D. & Gogoi, M. 2017. Nutrient composition of some nuts and oilseeds based recipes of Assam, India. *Journal of Human Ecology*, 18, 161–165.

Ewing, W. N. 1997. *The feeds directory* : vol. 1, Commodity products. Coalville: Context, p. 118.

Gálik, B., Šimko, M., Juráček, M., Bíro, D., Horniaková, E., Rolinec, M., Pastierik, O., Kolesárová, A., Maiorano, G., Gambacorta, M., Tavaniello, S. & Bednarczyk, M. 2011. *Biotechnology and animal food quality*. 1st ed., Nitra: Slovak University of Agriculture, p. 130.

Gálik, B., Bíro, D., Šimko, M., Juráček, M., Horniaková, E. & Rolinec, M. 2016. *Nutritional characteristics of feed*. 1st ed., Nitra: Slovak University of Agriculture, p. 101.

Geremew, A. – Getahun, A. & Rana, K. 2015. Digestibility of soybean cake, niger seed cake and linseed cake in juvenile Nile tilapia, *Oreochromis niloticus* L. *Journal of Aquaculture Research and Development*, 6(5), 333.

Goes, R. H. T., Souza, K. A., Patussi, R. A., Cornelio, T. C., Oliveira, E. R. & Brabes, K. C. S. 2010. Degradabilidade *in situ* dos grãos de crambe, girassol e soja, e de seus coprodutos em ovinos. *Acta Scientiarum. Animal Sciences*, 32, 271–277.

Gunstone, F. D. 2002. Production and trade of vegetable oils. In Gunstone, F. D.: *Vegetable oils in food technology composition, properties and use*. 1st ed. Oxford: Blackwell Publishing.

Ítavo, L. C. V., Soares, C. M., Ferraire Ítavo, C. C. B., Dias, A. M., Petit, H. V., Leal, E. S. & Vieira de Souza, A. D. 2015. Calorimetry, chemical composition and *in vitro* digestibility of oilseeds. *Food Chemistry*, 185, 219–225.

Leming, R. & Lember, A. 2005. Chemical composition of expeller-extracted and cold-pressed rapeseed cake. *Agraarteadus Journal of Agricultural Science*, 16(2), 96–103.

McKevith, B. 2005. Nutritional aspects of oil seeds. *British Nutrition Foundation Nutrition Bulletin*, 30, 13–26.

Morris, D. H. 2007. Flax – *A Health and Nutrition Primer*. 4th ed., Canada: Flax Council of Canada, p. 140.

Nadeem, M., Anjum, F. M., Arshad, M. U. & Hussain, S. 2010. Chemical characteristics and antioxidant activity of different sunflower hybrids and their utilization in bread. *African Journal of Food Science*, 4(10), 618–626.

Petrikovič, P., Sommer, A., Čerešňáková, Z., Svetlanská, M., Chrenková, M. , Chrastinová, Ľ., Poláčiková, M., Bencová, E. & Dolešová, P. 2000. Nutritional value of feeds, II. part. 1st ed., Nitra: Research Institute of Animal Production, Nitra, Slovakia, p. 280.

Ramachandran, S., Singh, S. K., Larroche, CH., Soccol, C. R. & Pandey, A. 2007. Oil cakes and their biotechnological applications: A review. *Bioresource Technology*, 98, 2000–2009.

Rymer, C. & Short, F. 2003. The nutritive value for livestock of UK oilseed rape and rapeseed meal. *Research Review*, no. Os14, p. 44.

Santos, V. C., Ezequil, J. M. B. & Oliveira, P. S. N. 2009. Consumo e digestibilidade em ovinos alimentados com grãos e subprodutos da canola. *The Brazilian Journal of Animal Health and Production*, 10, 96–105.

Van Eys, J. E. 2004. *Manual of Quality Analysis for Soybean Products in the Feed Industry*. 2nd ed., U. S. Soybean Export Council. http://ussec.org/wp-content/uploads/2012/09/Manual-of-Quality-Analyses-2nd-edition.pdf

Zeman, L. 2006. *Nutrition and feeding of farm animals*. 1st ed., Praha: Profi Press., p. 360.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 52, 2019 (1): 16–23

ISSN 1337-9984
E-ISSN 1338-0095

PERFORMANCE, APPARENT DIGESTIBILITY AND NITROGEN UTILIZATION BY WEST AFRICAN DWARF EWES FED ENSILED *ALTERNANTHERA BRASILIANA* (L.) O *KUNTZE* BASED DIETS

Victor Olusegun AKINWANDE*, Adejoke Adeneye MAKO, Saidat BAKARE, Lateefat SIKIRU, Bukola TAIWO

Department of Agricultural Science Tai Solarin University of Education, Ijagun, Ijebu-Ode, Ogun State, Nigeria

ABSTRACT

The objective of this study was to evaluate the performance, apparent nutrient digestibility and nitrogen utilization by West African Dwarf (WAD) ewes fed ensiled *Alternanthera brasiliana* (AB) based diets. The chemical composition of ensiled AB was determined. Performance, apparent nutrient digestibility and nitrogen utilization by WAD ewes were also determined. Thirty (30) female WAD ewes between the ages of 1 – 1.5 years, weighing 9.18 – 11.8 kg were used for feeding trial, which lasted for 56 days. In a completely randomized design, six animals were allotted to each of five diets comprising of 0 %, 30 %, 45 %, 60 %, and 90 % ensiled AB inclusion level. Parameters measured include: dry matter intake, growth rate, feed conversion, apparent digestibility of dry matter and crude protein, nitrogen intake, urinary nitrogen, nitrogen balance and retention. Results revealed that, the dry matter of ensiled AB was 80.38 %, the crude protein and neutral detergent fibre were 18.38 % and 61.51 % respectively. The quality characteristic of ensiled AB was optimal as the aroma, colour, texture, temperature (28.0 ˚C) and pH (4.05) indicated fermentation and good keeping quality. Significant variations ($p < 0.05$) occurred in the dry matter intake, daily body weight gain and feed conversion ratio. They ranged from 793.54 – 1020.25 $g.d^{-1}$; 53.57 – 98.21 $g.d^{-1}$ and 10.39 to 14.81 $g.d^{-1}$ in ewes fed 0 % and 90 % AB inclusion level. Significant differences were observed in dry matter, crude protein, crude fibre, ether extract, ash and nitrogen free extract digestibility. The values ranged from 89.01 – 93.95 %, 92.00 – 98.11 %, 80.11 – 95.02 %, 92.10 – 99.00 %, 82.21 – 96.01 % and 51.31 – 89.02 % respectively. The nitrogen balance and nitrogen retention also differed significantly. They ranged from 18.26 – 23.69 $g.d^{-1}$ and 91.70 – 97.80 % in ewes that consumed 30 % and 90 % ensiled AB respectively. It was observed that the ewes on diet of 90 % ensiled AB performed optimally and better than ewes on other diets. It can be concluded that ruminant animals can survive and perform optimally on ensiled AB alone, especially during the off season in the tropics.

Key words: *Alternanthera brasiliana* silage; feed utilization; nutrient profile; sheep

INTRODUCTION

In Nigeria, low animal protein intake has remained a major nutritional problem, especially for low income and non-wage earners (Adeojo *et al*., 2014). Atsu (2012), reported that animal source of protein is expected to contribute 35 g per head per day but the actual amount of protein from animal source is only 15 g per head per day which is grossly inadequate. This has called for the identification of lesser known feed resources, which are capable of boosting the performance of livestock.

Nutrition in terms of quantity and quality is one of the ways to enhance the productivity of livestock to produce animal protein available for human growth and development (Babayemi,

***Correspondence:** E-mail: akinwandevo@tasued.edu.ng
Akinwande Victor, Associate Professor, Department of Agricultural Science
Tai Solarin University of Education, Ijagun, Ijebu-Ode, Ogun State, Nigeria
PMB 2811 Ijebu-Ode
Tel.: +2348036708218 & +2348137062856

Received: January 25, 2018
Accepted: April 26, 2018

2007). Research into cheaper and qualitative sources of livestock feedstuff to replace the expensive conventional feedstuffs particularly those of energy and protein origin is gathering momentum. The limited supply of feed ingredients for livestock feed industry has resulted in a continuous increase in the cost of production. The long term survival of traditional livestock production system within the rapidly evolving national economy of Africa will depend on their capacity to provide products in quantities and at prices which satisfy the subsistence and income needs of livestock producers. Equally, the survival of these production systems will depend on the availability of feed materials in the right quantity and quality for animal intake.

Given the current pattern of small ruminant production, due to high cost of feeding livestock, resulting from competition between man and animals for the available feed ingredients and also the seasonal fluctuation in the availability of pasture (Babayemi, 2006), it is appropriate to consider the potential of lesser known feed materials as supplements in ruminant nutrition. The cost of feeding livestock is about 60 – 80 % of the total cost of production (Mako *et al*., 2012). The growth of livestock industry depends greatly on the progress that is made on the utilization of underutilized fodder as animal feed and the ability of these animals to convert such into animal products such as milk, meat, hides and skin.

AB is one of the lesser known plants that can be incorporated into the diets of ruminants. AB is a shrub, native to South America but has become naturalized in countries throughout the tropics, being found in Nigeria, Kenya, India, Ceylon, Cuba, Colombia (Sanchez-Del, 2012) and also in Laos, Vietnam and Cambodia. This study was designed to determine the chemical composition and keeping quality of ensiled AB and also to investigate the performance, apparent digestibility of nutrients and nitrogen utilization by WAD ewes fed ensiled AB.

MATERIAL AND METHODS

Experimental site

The experiment was carried out at the Tai Solarin Teaching and Research Farm, sheep and goat unit (7˚ 15' N to 7˚ 40' E). Each animal pen was made of walls of about 1 m high and each pen was about 220 cm long and 121 cm wide. The floor of the pen was made of concrete and the roof of the goat unit housing the pens was made of corrugated iron sheets. The pens were dusted and washed thoroughly with warm detergent to remove dirt. The pens were disinfected with broad spectrum insecticides, acaricides, and larvicides (diasuntol). The experimental animals were brought into the pens after 7 days of disinfection and they were allowed to acclimatize to the new environment for another 7 days before feeding trial began. Hence, there was a preparation period of 14 days.

Experimental animals

Thirty (30) adult female WAD ewes used for this experiment were randomly allotted to (five) 5 experimental diets with six (6) animals in each group. The animals were between 1 – 1.5 years of age, weighing 9.18 – 11.8 kg. The feeding trial lasted for 56 days.

Feeding trial

AB was harvested from a cultivated plot on the Teaching & Research Farm of Agricultural Science Department, Tai Solarin University of Education, Ijagun, Ijebu-Ode, Ogun State, Nigeria. The harvested plant was chopped and ensiled with wheat offal in ratio 80: 20 % w/w respectively for 42 days as per the procedure of Akinwande (2011). Polythene bags, each capable of holding at least 30 kg of AB were used as silos. Each polythene bag was then placed inside a 65 liter capacity plastic basin for reinforcement and ease of handling. Sealing of silos was effected by placing a 25 kg sand-bag on top of each polythene bag after tying carefully and timely. The ensiled AB was used to formulate five experimental diets fed to the animals.

Diet 1 = 90 % guinea grass + 0 % ensiled AB + 10 % concentrate
Diet 2 = 60 % guinea grass + 30 % ensiled AB+ 10 % concentrate
Diet 3 = 45 % guinea grass + 45 % ensiled AB + 10 % concentrate
Diet 4 = 30 % guinea grass + 60 % ensiled AB + 10 % concentrate
Diet 5 = 0 % guinea grass + 90 % ensiled AB + 10 % concentrate

Ensiled AB, Guinea grass and concentrate were fed at 0900 h each day, the refusal was weighed the following morning at 0800 h and deducted from the total amount of feed served the previous day for determination of feed intake. Daily feed was served to meet 5 % of the sheep's body weight (1 % of concentrate, 4 % of AB and Guinea

grass) and this was frequently adjusted to ensure that each animal received about 20 % of feed above its previous day's consumption. Feed refusal was sampled daily and mixed for the entire collection period on an individual basis using an air tight plastic bag. Samples from refusal were taken for proximate composition analysis. Fresh water was served each day; salt lick was placed permanently in each cage. The animals were weighed at the beginning and end of the feeding trial to determine weight gain.

Digestibility trial

Twenty (20) Ewes from the thirty (30) used for the growth study were randomly selected for determination of digestibility of the diets. The experiment lasted 14 days, for collection of feaces and urine. The animals were fed at 0900 hours daily. Feed was served at 5 % of the body weight of the animals. Water and salt lick were accessible to the animals throughout the metabolic period. Feed refused was weighed at 0800 hours every morning and deducted from the total feed offered the previous day, prior to serving new feed daily. Fresh water was also served *ad libitum*. During fourteen days of collection, total faeces was collected, weighed and 10 % aliquot was taken and stored in the freezer at -4 ˚C. After a 14-day collection period, the total faeces from daily collection were bulked, mixed and dried in the oven and kept till required for chemical analysis. Urine samples were collected and measured daily for each animal in the morning using measuring cylinder and kept in separately labeled containers. Two drops of concentrated sulfuric acid was added to each container daily after collection of each sample to prevent microbial growth and loss of the nitrogen measured. Approximately 10 % of total urine was sampled daily and stored at -4 ˚C till required for nitrogen analysis. Apparent nutrient digestibility was determined for crude protein, ether extract, crude fibre, ash and nitrogen free extract using the formula as follows:

$$\frac{\textit{Nutrient in feed} - \textit{nutrient in feaces}}{\textit{Nutrient in feed}} \times 100$$

Nitrogen utilization was also determined by analyzing the nitrogen content of the urine and feaces.

Chemical analysis

Crude protein, crude fibre, ether extract and total ash of experimental diets were analyzed in triplicates using standard procedure of A.O.A.C (2012). The crude protein was determined with the micro Kjeldahl distillation apparatus. Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) were determined according to Van Soest (1995).

Shown in Table 1 is the chemical composition of ensiled AB and *Panicum maximum*. The dry matter (DM) and crude protein (CP) obtained for ensiled AB in this study are 80.81 % and 18.38 % respectively. The values here are higher than values reported for water hyacinth ensiled with different additives (19.60 – 29.74 % and 10.76 – 16.23 %) for DM and CP respectively by Akinwande *et al*. (2014). These values are also at variance with the value range of 18.36 – 30.03 % for DM and 4.52 – 5. 50 % for CP reported for elephant grass ensiled with cassava peel, (Olorunnisomo and Dada, 2011). The CP obtained here is above the recommended level of 7 – 8 % that can provide the minimum requirement for microbial activities (NRC 2002). The value of Neutral detergent fibre of ensiled AB (61.51 %) is similar to the value reported for water hyacinth ensiled with different additives (Akinwande *et al.*, 2014). The CP obtained here is within the recommended value that will enhance dry matter intake (Wanapat *et al*., 2013).

Table 1. Chemical composition (%) of *Alternanthera brasiliana* (AB) ensiled with wheat offal and *Panicum maximum* (PM)

Parameters	AB	PM
Dry matter	80.38	90.35
Crude protein	18.38	8.43
Ether extract	3.54	10.93
Ash	9.84	13.32
Neutral detergent fibre	61.51	71.21
Acid detergent fibre	46.89	48.70

Table 2 presents the quality characteristics of AB ensiled with wheat offal for 42 days. The colour, texture, odour, temperature and pH of ensiled AB are similar to the findings of Akinwande *et al.*, (2011) for water hyacinth ensiled with different

Table 2. Quality characteristics of *Alternanthera brasiliana* ensiled with wheat offal

Silage	Colour	Texture	Odour	Temperature (˚C)	pH
AB ensiled with Wheat offal	Khaki brown	Firm	Pleasant alcoholic	28.00	4.05

additives. The results obtained here indicated that fermentation occurred considering the pH value of 4.05 which could infer good quality of the silage that would be well preserved. A properly fermented silage will have a much lower pH (i.e. be more acidic) and pH is one of the simplest and quickest ways of evaluating silage (Babayemi, 2009). Any silage with pH ranges of 4.5 – 5.5 or 4.3 – 4.7 has been classified to be good (Meneses, 2007; Kung and Shaver, 2002) respectively. The temperature obtained here is in agreement with other studies elsewhere (Akinwande, 2011). This would appear to be a good operating temperature for silage fermenting organisms.

Statistical analysis

Data obtained were analyzed and subjected to analysis of variance procedure (ANOVA) of SAS (2012). Significant means were separated by Duncan's Multiple Range Test of the same statistical package.

RESULTS AND DISCUSSION

Table 3 presents the feed intake and performance of WAD ewes fed ensiled AB-based diets. Results revealed N dry matter increased as the test material increased. The dry matter intake ($g.d^{-1}$) ranged significantly ($P < 0.05$) from 793.24 – 1020.25 in ewes fed 0 % and 90 % AB inclusion level respectively. This result is in agreement with the findings of Adegbola *et al*., (1987), who reported a range of 624.11 – 1044.24 $g.d^{-1}$ for dry matter intake for WAD sheep fed processed cassava peel with *Gliricidia sepium*. This result is higher and at variance to the value range of 628 – 798.5 $g.d^{-1}$ reported for WAD sheep fed *Atriplex halimus* ensiled with three developed enzyme cocktails (Salem *et al*., 2015) and 573.83-715.14 $g.d^{-1}$ reported for WAD rams fed *Panicum maximum* ensiled with two cultivars of *Lablab purpureus* (Alasa, 2014). It was observed that ewes on 90 % AB

Table 3. Feed intake and performance of WAD Ewes fed ensiled *Althernanthera brasiliana* based diets

Parameters	Level of ensiled *Alternanthera brasiliana* Inclusion					SEM
	0 %	30 %	45 %	60 %	90 %	
Intake (g/DM/day)						
Concentrate	95.20^{a}	90.27^{b}	89.20^{d}	88.20^{d}	88.92^{c}	0.51
Guinea grass	698.40^{a}	259.70^{b}	210.90^{c}	166.8^{d}	-	12.70
Ensiled AB	-	488.00^{d}	541.50^{c}	706.20^{b}	931.3^{a}	10.20
Total dry matter intake ($g.d^{-1}$)	793.24^{e}	838.10^{d}	842.29^{c}	961.20^{b}	1020.5^{a}	22.10
Initial body weight (kg)	12.00	11.00	10.50	10.50	10.50	0.70
Final body weight (kg)	15.00^{c}	15.00^{c}	14.50^{d}	15.50^{b}	16.00^{a}	0.33
Body weight gain (kg)	3.00^{d}	4.00^{c}	4.50^{b}	5.00^{b}	5.50^{a}	0.25
Daily body weight gain ($g.d^{-1}$)	53.57^{d}	71.43^{c}	71.43^{c}	89.30^{b}	98.21^{a}	7.89
Feed conversion ratio	14.81^{a}	11.73^{b}	11.79^{b}	10.76^{c}	10.39^{d}	0.91

$^{a, b, c, d, e}$ = means on the same row with different superscript differed significantly ($p < 0.05$)

SEM = standard error of mean.

Formular for feed conversion ratio: $\frac{\text{Dry matter intake (g)}}{\text{Daily weight gain (g)}}$

inclusion level recorded the highest (1020.25 g.d^{-1}) dry matter intake, while the lowest dry matter intake (793.54 g.d^{-1}) was recorded for ewes fed 0 % AB inclusion level. The higher intake of silages may be dues to the sweet and pleasant acid (Lactic Acid) aroma of the plant (Babayemi, 2009). Morinson (1959) pointed out that silage even from plants with coarse stalks such as corn and sorghums are eaten practically without waste. The daily body weight gain also ranged significantly from 53.57 to 98.21 g.d^{-1} in ewes on 0 % and 90 % AB diets respectively. This result is at variance with the report elsewhere for sheep fed ensiled water hyacinth (Akinwande *et al*., 2014) but similar and in agreement with the findings of Adegbola *et al*., (1987). The higher weight gain of ewes on 60 % and 90 % AB diets could be attributed to the higher and rapid by-pass protein from the rumen and subsequent digestion and absorption in the abomasum and duodenum. The 30 % and 45 % AB diets may have stayed longer in the rumen and are utilized by the microbes for single cell formation. Belanche *et al*., (2017) reported that microbial colonization of highly lignified particles is limited. Ewes fed 60 % and 90 % AB diets consumed more than 3 % of their body weights, which agrees with the value of 3 – 5 % body weight as DMI recommended for ruminants (NRC 2002). The feed conversion ratio also ranged significantly (P < 0.05) from 10.39 to 14.81 in ewes fed 90 % and 0 % AB diets respectively. The ewes on 90 % AB diet had the best daily weight gain compared to ewes on other diets due to the least feed conversion ratio it had, this is in accordance with Smeaton (2003), who opined that the lower the feed conversion ratio value, the more efficient the animals converted the feed to meat.

Table 4 shows the apparent digestibility of nutrients by WAD ewes fed ensiled AB. The digestibility values differed significantly (P < 0.05) among the treatment means. The digestibility for dry matter ranged from 89.01 to 93.95 % in ewes fed 0 % and 90 % ensiled AB respectively. This result is lower and at variance with the value range of 54.67 – 68.00 % reported for Red Sokoto goats fed elephant grass ensiled with cassava peel (Olorunnisomo and Dada, 2011). The crude protein digestibility value for ewes on 90 % AB diets was significantly higher (P < 0.05) than those on 0 % AB diet (96.10 %) and 60 % AB diet (97.20 %) whose values differ significantly (P < 0.05) from each other and this is an index of microbial protein made available to the ewes daily (Kissada *et al*., 2010). This result is higher than the values reported for goats fed elephant grass ensiled with cassava peel (Olorunnisomo and Dada 2011). The crude fibre digestibility followed the same trend with crude protein digestibility. However, the digestibility of DM, CP and CF are in agreement with the digestibility obtained for goats fed sun-cured water hyacinth (Mako, 2009).

Presented in Table 5 are nitrogen utilization, nitrogen balance and retention. These are functions of nitrogen ingested and digested; they varied significantly (P < 0.05) among treatment means with ewes on 90 % AB diet recording the highest (23.69 g.d^{-1} and 97.80 %), while ewes on 30 % AB diet recorded the lowest (18.26 g.d^{-1} and 91.70 %). The nitrogen retention values obtained in this study are similar to value range reported for kids

Table 4. Apparent digestibility (%) by WAD Ewes fed ensiled *Alternanthera brasiliana*

Parameters	Level of ensiled *Alternanthera brasiliana* Inclusion					SEM
	0 %	30 %	45 %	60 %	90 %	
Dry matter	89.01^{e}	90.21^{d}	90.35^{c}	92.51^{b}	93.95^{a}	1.01
Crude protein	96.10^{c}	92.00^{d}	92.10^{d}	97.20^{b}	98.11^{a}	2.11
Ether extract	95.01^{d}	99.00^{a}	92.10^{d}	96.10^{c}	98.12^{b}	2.35
Crude fibre	89.12^{c}	80.11^{d}	80.11^{d}	92.01^{b}	95.02^{a}	2.04
Ash	91.01^{c}	82.21^{e}	83.21^{d}	93.02^{b}	96.01^{a}	1.00
Nitrogen free extract	72.11^{c}	51.31^{e}	53.21^{d}	82.11^{b}	89.02^{a}	1.15

$^{a, b, c, d, e}$ = means on the same row with different superscript differed significantly (p < 0.05)
SEM = standard error of mean.

Table 5. Nitrogen utilization by WAD Ewes fed ensiled *Alternanthera brasiliana*

Parameters	Level of ensiled *Alternanthera brasiliana* Inclusion					SEM
	0 %	30 %	45 %	60 %	90 %	
Nitrogen intake ($g.d^{-1}$)	19.050^{e}	19.910^{d}	20.010^{c}	22.840^{b}	24.230^{a}	0.050
Feacal nitrogen ($g.d^{-1}$)	0.580^{c}	1.600^{b}	1.650^{a}	0.740^{d}	0.540^{e}	0.020
Urinary nitrogen ($g.d^{-1}$)	0.034^{b}	0.050^{a}	0.014^{c}	0.010^{d}	0.004^{e}	0.001
Total nitrogen excreted ($g.d^{-1}$)	0.614^{c}	1.650^{a}	1.660^{a}	0.750^{b}	0.544^{d}	0.001
Nitrogen balance ($g.d^{-1}$)	18.440^{c}	18.260^{d}	18.350^{e}	22.090^{b}	23.690^{a}	0.020
Nitrogen retention (%)	95.700^{c}	91.700^{d}	91.700^{d}	96.720^{b}	97.800^{a}	2.010

$^{a, b, c, d}$ = means on the same row with different superscript differed significantly ($p < 0.05$)
SEM = standard error of mean.

fed varying proportions of Zinc (Osineye, 2011), however, these value ranges are higher and at variance with the range (9.86 – 29.80 %) reported for Red Sokoto goats fed varying levels of energy source (Otaru *et al*., 2011), also higher than 62.5 – 74.4 % reported for goats fed ensiled maize stover and supplemented with *Bobgunnia madagascariensis* (Kanyinji *et al*, 2017). The positive nitrogen balance obtained may be indicative of proper utilization of the silage.

CONCLUSION

Ensiling AB with wheat offal had beneficial effects on silage qualities, intake and performance of WAD ewes. AB is a shrub that is readily available in the tropics especially during the rainy season. It can be preserved to feed animals during the dry season. It can then be concluded from the result of this study that ruminant animals can survive and perform optimally on ensiled AB alone.

REFERENCES

Adegbola, A. A., Smith, O. B. & Asaolu, V. O. 1987. *Response of West African Dwarf Sheep to diets based on processed cassava peel and Gliricidia sepium*. FAO Corporate document Repository. Produced by ILRI. www.fao.org/wairdocs/ilri/x5490e/x5490e0m.htm

Adeojo, P. E., Mohammed, I. A. & Alus, F. S. 2014. Analyzing consumer's preference for snails to other meats in Kabsba/Bunu L.G.A of kogi state Nigeria. *American Journal of Food Science and Health*, 2(4), 82–87.

Akinwande, V. O. 2011. *Utilization of ensiled water hyacinth* (*Eichhornia crassipes* Mart. Solms-Laubach) *by West African Dwarf Sheep*. PhD Thesis. Department of Animal Science University of Ibadan.

Akinwande, V. O., Mako, A. A. & Babayemi, O. J. 2011. *Silage quality, voluntary feed intake (VFI), Nutrient digestibility and Nitrogen Balance in WAD sheep fed ensiled water hyscinth (WH) in Nigeri*a. Proceedings of the 36th Animal Production (NSAP) held at Development of Animal Science, Faculty of Agriculture University of Abuja between 13th – 16th March, 509–512.

Akinwande, V. O., Mako, A. A. & Babayemi, O. J. 2014. *Performance characteristics of WAD sheep fed water hyacinth silage fermented with different Agro-industrial additive*s. Proceedings of the 39th Annual conference of Nigerian society for Animal Production (NSAP) held at Babcock University ilishan between 16th and 19th March, 384–387.

Alasa, M. C. 2014. *Nutritional Assessment of Panicum maximum* (Jacq.) *ensiled with two* cultivars of *Lablab purpureus* (*Lablab purpureus* L.) *for West African Dwarf Ram*. PhD Thesis. Department of Animal Science. University of Ibadan.

AOAC. 2012. *Official methods of Analysis*, 19th ed. Association of Officials Analytical Chemists, Arlington, VA.

Atsu, D. W. 2012. Contributory role of Animal production in National Development Process of the 17th Annual Conference of Animal Science Association of Nigeria. September 16th–19th 2012.

Babayemi, O. J. 2006. Evalution of the nutritive value and free choice intake of two aquatic weeds (*Nephrolepis biserratha* and *Spirodels polyrhiza*) by West African Dwarf goats. *Tropical and Subtropical Agroecosystems*, 6 (1), 15–22.

Babayemi, O. J. 2007. *In Vitro* Fermentation Characteristics and Acceptability by West African dwarf Goats of some Dry Season Forages. *African Journal of Biotechnology*, 6(10), 1260 –1265.

Babayemi, O. J. 2009. Silage quality, dry matter intake and digestibility by West African dwarf sheep of Guinea grass (*Panicum maximum* cv Ntchisi) harvested at 4 and 12 week re-growths.*African Journal of Biotechnology*, 8, 3983–3988.

Belanche, A., Newbold, C. J., Lin, W., Ree-Stevens, P. & Kingston-Smith, A. H. 2017. A systems Biology approach reveals differences in the dynamics of colonization and degradation of grass Vs Hay by rumen microbes with minor effects of vitamin supplementation. *Front Microbiology*, 8, 1458.

Kanyinji, F., Nguni, M. & Mulenga, A. 2017. Intake, digestibility and nitrogen retention in goats fed ensiled maize stover and supplemented with snake bean (*Bobgunnia madagascariensis*) pod meal. *Journal of Advanced Veterinary and Animal Research*, 4(2), 168–174.

Kissada, B., Metha, W. & Chainarong, N. 2010. Replacement of soybean meal by yeast fermented-cassava chip protein (YEFECAP) in concentrate diets fed on rumen fermentation, microbial population and nutrient digestibility in ruminants. *Journal of Animal and Veterinary Advances*, 9, 1727–1734.

Kung, L. & Shaver, R. 2002. *Interpretation and use of silage fermentation analyses reports*. Department of Animal and Food Science, University of Delware Newark, DE 19717.

Mako, A. A 2009. *Evaluation of water hyacinth* (*Eichhornia crassipes* Mart. Solms-Laubach) *as a potential feed for the West African Dwarf goats.* PhD Thesis. Department of Animal Science. University of Ibadan.

Mako, A. A., Akinwande, V. O., Abiola-Olagunju, O. I., Babayemi, O. J. & Akinsoyinu, A. O. 2012. Variations in Chemical Nutrients and Mineral Concentrations of Water Hyacinth (*Eichhornia crassipes* Mart. Solms-Laubach). *Nigerian Journal of Animal Production*, 39(1), 199–205.

Meneses, M. D., Megias, J., Madrid, A., Martinez-Teruel, F., Hernadez, J. & Oliva, J. 2007. Evaluation of the Phytosanitary, Fermentative and nutritive characteristics of silages made from crude artichoke (*Cynara scolymus* L.) by- Product feeding for ruminants. *Small Ruminant Research*, 70, 292–296.

Morrison, F. B 1959. *Feeds and feeding*. Morrison Publishing Company, New York.

NRC. 2002. *National Research council. Publicity Funded Agricultural Reseach and the changing structure of U.S Agriculture*. Washington, DC: The National Academic Press. http.//doi.org/10.17226/10211

Olorunnisomo, O. A. & Dada, O. A. 2011. *Digestibility and intake of elephant grass ensiled with cassava peels by Red Sokoto goats in Ibadan*. Proceedings of the 36th Annual conference of Nigeria Society for Animal Production. Held between 13-16th March 2011 at Merit House/ Raw Materials Research and Development Council, Abuja, pp. 584–586.

Osineye, O. M. 2011. *Zinc on energy and nitrogen utilization in West African Dwarf Kids.* Proceedings of the 36th Annual conference of Nigeria Society for Animal Production. Held between 13-16th March 2011 at Merit House/ Raw Materials Research and Development Council, Abuja, pp. 587–590.

Otaru, S. M., Adamu, A. M., Ehoche, O. W. & Makun, H. J. 2011. *Effect of varying the level of energy source on digestibility of nutrients and nitrogen retention in Red Sokoto goats.* Proceedings of the 36th Annual conference of Nigeria Society for Animal Production. Held between 13-16th March 2011 at Merit House/ Raw Materials Research and Development Council, Abuja, pp. 591–594.

Salem, A. Z. M., Alsersy, H., Camacho, L. M., EL -Adawy M. M., Elghandour, M. M. Y., Kholif, A. E., Rivero, N., Alonso, M. U. & Zaragoza, A. 2015. Feed intake nutrient digestibility, nitrogen utilization, and ruminant fermentation activities in sheep fed Atriplex halimus ensiled with three developed enzyme cocktails. *Czech Journal of Animal Science*, 4, 185–194.

SAS. 2012. *Statistical Analytical Systems*. SAS Version 9.2 user's guide. Carry, NY: SAS Institute.

Sanchez-Del, P. 2012. Molecular phytogenetics of *Alternanthera* (Gomphrenoidae, Amaranthaceae): resolving complex taxonomic history caused by different interpretations of morphological characters

in a lineage with C_4 and C_3-C_4 intermediate species. *Botanical Journal of the Linnean Society*, 169(3), 493–517.

Smeaton, D. C. 2003. *Profitable Beef Production: A Guide to Beef Production in New Zealand.* Published by the New Zealand Beef Council.

Van Soest, P. J. 1994. Nutritional Ecology of Ruminants, 2nd ed. Cornell University Press.

Wanapat, M., Pilajun, R., Polyorach, S., Cherdthrong, A., Khejornsart, P. & Rowlinson, P. 2013. Effect of Carbohydrate sources and Cotton- seed meal level on feed intake, nutrient digestibility, Rumen fermentation and Microbial Protein Synthesis in swamp Buffaloes. *Asian Australas Journal of Animal Science*, 26(7), 952–960.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 52, 2019 (1): 24–29

ISSN 1337-9984
E-ISSN 1338-0095

CELL RECEPTORS MEDIATING COMMUNICATION BETWEEN PREIMPLANTATION EMBRYO AND SURROUNDING ENVIRONMENT: CLUES FROM MOUSE AND RABBIT MODELS: A REVIEW

Štefan ČIKOŠ*, Alexandra ŠPIRKOVÁ, Janka KUBANDOVÁ, Veronika KOVAŘÍKOVÁ, Zuzana ŠEFČÍKOVÁ, Dušan FABIAN, Juraj KOPPEL

Centre of Biosciences of the Slovak Academy of Sciences, Institute of Animal Physiology, Košice, Slovakia

ABSTRACT

Preimplantation period of embryo development is one of the most sensitive phases in the mammalian ontogeny, and disturbances at this developmental stage can result in poor pregnancy outcomes (in both embryos resulting from natural conception or from biotechnology procedures). Results of experimental studies have shown that maternal physiological conditions and external environmental factors can significantly influence preimplantation embryo development, indicating a communication between the early embryo and its environment. The study of communication between the early embryo and surrounding environment has been focused mainly on protein signaling molecules, such as growth factors and cytokines. However, small-molecule ligands, such as biogenic monoamines, have been shown to influence preimplantation embryo development as well, and results obtained on mouse and rabbit models indicate that biogenic monoamine receptors are expressed in preimplantation embryos. Several adrenergic, dopamine, serotonin and histamine receptors were detected in mouse and rabbit ovulated oocytes and preimplantation embryos, and in mouse embryonic stem cells. Although the physiological role of biogenic monoamine receptors in early embryonic cells is not fully understood, experimental data indicate their involvement in the regulation of cell proliferation, differentiation and survival under physiological as well as unfavorable or pathological conditions (e.g. during maternal stress).

Key words: preimplantation embryo; cell receptors; embryo-maternal communication

INTRODUCTION

An important part of animal biotechnology is focused on animal reproduction, and number of approaches, such as artificial insemination, multiple ovulations, *in vitro* fertilization, embryo culture, embryo transfer, cryopreservation of gametes and embryos, nuclear transfer or cloning, transgenesis and embryonic stem cell production, are used in this field. Fertilized oocyte (zygote), which can result from natural conception or artificial insemination, develops several days in the oviduct, and finally it implants into the uterine wall at the blastocyst stage. In some assisted reproductive technologies, the preimplantation development takes place *in vitro* and the blastocyst is then transferred into the uterus. Alternatively, the inner cell mass of blastocysts can serve as a source of embryonic stem cells, which are potentially usable in regenerative medicine.

Although the preimplantation period of development lasts only for a relatively short time, it represents one of the most sensitive phases in mammalian ontogeny (up to 50 % of embryo loss occurs during this period), and disturbances at this developmental stage can result in poor

***Correspondence:** E-mail: cikos@saske.sk
Štefan Čikoš, Centre of Biosciences of the Slovak Academy of Sciences, Institute of Animal Physiology, Šoltésovej 4-6, 04001, Košice, Slovakia

Received: November 20, 2018
Accepted: December 3, 2018

pregnancy outcomes in both humans and animals (Nepomnaschy *et al*., 2006; Humblot, 2001; Diskin *et al*., 2008). The preimplantation embryo can develop relatively autonomously in very simple culture media, utilizing transcripts and proteins provided by the oocyte and gene products synthesized by an embryo itself (after activation of the embryonic genome). However, there are data indicating that *in vitro* culture of preimplantation embryos can alter gene expression and epigenetic reprogramming, and can lead to abnormal fetal and perinatal growth as well as to physiological and behavioral alterations in adulthood (for review see Khosla *et al*., 2001; Fleming *et al*., 2004; Ventura-Junca *et al*., 2015). Moreover, results of animal studies and observations in humans have shown that maternal physiological condition (nutritional status, body condition, metabolic and other disorders, stress) as well as external environmental factors, e.g. various xenobiotics that can contaminate food, can significantly influence preimplantation embryo development (Kwong *et al*., 2000; von Borell *et al*., 2007; Fabian *et al*., 2010; Kubandová *et al*., 2014; Burkuš *et al*., 2013; 2015; Janštová *et al*., 2017; Babeľová *et al*., 2017). These data indicate that communication between the embryo and its environment takes place already in very early developmental stages.

The study of communication between the early embryo and surrounding environment has been mainly focused on protein signaling molecules, such as growth factors and cytokines, and relatively many receptors for protein ligands have been identified in preimplantation embryos (for review see Hardy and Spanos, 2002; Thouas *et al*., 2015). However, we and others have demonstrated that small-molecule ligands, such as biogenic monoamines, can significantly influence preimplantation embryo development as well. This minireview focuses on the expression of receptors for biogenic monoamines and their potential role in early embryo development.

Laboratory mouse is the most widely used model for the study of early mammalian embryo development and most information on mechanisms involved in embryo-maternal communication comes from the mouse model. Recently, the progress in rabbit genome sequencing and advantages of rabbit reproduction (high cell numbers and yield in blastocysts, relatively late implantation at a time when gastrulation is already proceeding, detailed morphologic and molecular knowledge on gastrulation stages and a hemochorial placenta structured similarly to the human placenta) are the reasons for the preferred use of a laboratory rabbit as a model for studying early embryo development (for review see Fischer *et al*., 2012). Results obtained mainly on mouse and rabbit models are presented in this review.

Receptors for biogenic monoamines

Biogenic monoamine receptors are either ion channels or G protein-coupled receptor proteins. Receptor channels (ligand-gated ion channels) are composed of several transmembrane subunits, and binding the ligand induces opening of the channel. G protein-coupled receptors are composed of seven transmembrane domains connected by cytoplasmic and extracellular loops. Binding the ligand results in activation of GTP-binding proteins (G proteins), which then activate various effectors, such as adenylyl cyclases, phospholipase C beta, and other proteins involved in signal transduction (for review see Krauss, 2014).

Most information on the expression of biogenic monoamine receptors during preimplantation development period has been obtained on mouse and rabbit models. Several catecholamine (adrenergic and dopamine) serotonin and histamine receptors were detected in mouse and rabbit ovulated oocytes and preimplantation embryos (see Table 1). Some biogenic monoamine receptors were detected in oocytes and preimplantation embryos of other species as well (Čikoš *et al*., 2014; Amireault and Dubé, 2005).

Embryonic stem cells (ESCs), derived from the inner cell mass of a blastocyst, can serve as an experimental model for studying early development. Although ESCs retain the high developmental potency of the founder embryo cells, several differences in the gene expression between preimplantation embryos and ESCs cells have been identified (Tanaka *et al*., 2002; Tang *et al*., 2010). Expression of catecholamine receptors has been examined in several mouse embryonic stem cell lines, and, in contrast to mouse preimplantation embryos, transcripts of all types of dopamine and adrenergic receptors were detected in mouse ESCs (Lee *et al*., 2006; Kim *et al*., 2008; Layden *et al*., 2010; Čikoš *et al*., 2015).

Differences in the expression of some adrenergic receptors between the spontaneously differentiating ESCs and undifferentiated ESCs indicate a role of these receptors in the process of embryonic stem cell differentiation (Čikoš *et al*., 2015). Interestingly, catecholamine receptor types that couple primarily to G proteins with opposite actions on adenylyl cyclase activity (beta adrenoceptors, DR1 and DR5 stimulate adenylyl cyclase activity, and alpha 2 adrenoceptors, DR2, DR3 and DR4 inhibit adenylyl cyclase activity) are expressed in mouse and rabbit preimplantation embryos and in the mouse embryonic stem cells, indicating a cross-talk between signaling pathways activated by these receptors.

The role of biogenic monoamines in early embryo development

Experimental data indicate that biogenic monoamines can (besides their well-known neurotransmitter function) play an important role in basic developmental processes, such as embryogenesis and morphogenesis (for review see Pendleton *et al*., 1998; Herlenius and Lagercrantz, 2001), and several biogenic monoamines have been identified in reproductive fluids of various mammalian species. For instance, adrenaline and noradrenaline have been detected in rabbit and bovine oviductal fluid and their concentrations varied with the region of the oviduct and the stage of the estrous cycle (Khatchadourian *et al*., 1987; Way *et al*., 2001).

Although the physiological role of biogenic monoamine receptors in early („pre-neural“) embryogenesis is mostly unknown, experimental data indicate that these receptors are functional and can be activated by agonists. Studies in mouse embryonic stem cells demonstrated that activation of dopamine and adrenergic receptors can trigger various signaling pathways influencing DNA synthesis and proliferation (Lee *et al*., 2006;

Table 1. Receptors of biogenic monoamines detected in mouse and rabbit ovulated oocytes and preimplantation embryos

Receptor	Species	Stage (mRNA)	Stage (protein)	Reference
H2R	mouse	Blast	Blast	Zhao *et al.*, 2000
5-HT_{1D}	mouse	Ooc, Zyg, 2-cell, 8-16-cell, Blast	not tested	Veselá *et al*., 2003 Iľková *et al*., 2004
5-HT_7	mouse	Ooc, Zyg, 2-cell, 4-cell	Ooc, 4-cell	Amireault & Dubé 2005
α1B-AR	rabbit	Ooc	not tested	Čikoš *et al*., 2014
α2A-AR	rabbit	Ooc, Morul, Blast	not tested	Čikoš *et al*., 2014
α2C-AR	mouse	Ooc, 8-16-cell, Blast	Ooc, 8-16-cell, Blast	Čikoš *et al*., 2007
	rabbit	Ooc, Morul, Blast	not tested	Čikoš *et al*., 2014
β1-AR	rabbit	Ooc, Morul, Blast	not tested	Čikoš *et al*., 2014
β2-AR	mouse	Ooc, 4-cell, 8-16-cell, Blast	Ooc, 4-cell, 8-16-cell, Bl	Čikoš *et al*.,2014 Chen *et al*., 2011
	rabbit	Ooc, Morul, Blast	not tested	Čikoš *et al*., 2014
β3-AR	mouse	Ooc, 4-cell, 8-16-cell, Blast	not tested	Čikoš *et al*., 2005
DR1	mouse	Blast	not tested	Čikoš *et al*., 2015
DR2	mouse	Ooc, 8-16-cell, Blast	not tested	Čikoš *et al*., 2015
DR3	mouse	Ooc, 4-cell, 8-16-cell, Blast	not tested	Čikoš *et al*., 2015
DR4	mouse	4-cell, 8-16-cell, Blast	not tested	Čikoš *et al*., 2015
DR5	mouse	Ooc, 4-cell, Blast	not tested	Čikoš *et al*., 2015

Receptors: H2R, histamine receptor subtype 2; 5-HT_{1D}, serotonin receptor subtype 1D; 5-HT_7, serotonin receptor subtype 7; 5-HT_{2A}, serotonin receptor subtype 2A; α1B-AR, adrenergic receptor subtype alpha 1B; α2A-AR, adrenergic receptor subtype alpha 2A; α2C-AR, adrenergic receptor subtype alpha 2C; β1-AR adrenergic receptor subtype beta 1; β2-AR, adrenergic receptor subtype beta 2; β3-AR adrenergic receptor subtype beta 3; DR1-5, dopamine receptor subtypes 1-5.
Developmental stages: Ooc, unfertilized oocytes (metaphase II stage); Zyg, fertilized oocytes (zygotes); 2-cell, two-cell embryos 4-cell, four-cell embryos; 8-16-cell, eight- to sixteen- cell embryos; Morul, morulas; Blast, blastocysts.

Kim *et al*., 2008; Sun *et al*., 2015). Most catecholamine receptors can regulate the intracellular cyclic adenosine monophosphate (cAMP) level, and it has been demonstrated that the cAMP signaling pathway can contribute to the regulation of mouse embryonic stem cell selfrenewal and differentiation (Faherty *et al*., 2007; Layden *et al*., 2010). Results of *in vitro* and *in vivo* experiments showed that catecholamine and serotonin receptors can activate signaling pathways regulating cell proliferation and survival in mouse preimplantation embryos (Markova *et al*., 1990; Čikoš *et al*., 2005; 2007; Veselá *et al*., 2003; Iľková *et al*., 2004). Moreover, there are data suggesting that catecholamine receptors (embryonic or uterine) can participate in the process of blastocyst implantation (Henriquez *et al*., 2006; Chen *et al*., 2011). The involvement of histamine receptor subtype 2 (regulating cAMP level) in the process of embryo implantation has been documented in the mouse model as well (Zhao *et al*., 2000). Results of *in vivo* experiments obtained on the mouse model indicate that catecholamine receptors together with glucocorticoid receptors could mediate effects of maternal stress on early embryo (Burkuš *et al*., 2013; 2015; Janštová *et al*., 2017; Zheng *et al*., 2016; Tan *et al*., 2017), and that expression of catecholamine receptors in early embryos can be influenced by maternal physiological status (Seeling *et al*., 2017).

CONCLUSION

Mammalian preimplantation embryo is equipped with a variety of cell receptors indicating active communication between the early embryo and its environment. Results obtained in mouse and rabbit models indicate that except of receptors binding protein ligands, the receptors that bind small-molecule ligands, such as biogenic monoamines, are expressed in preimplantation embryos and in the embryonic stem cells derived from mouse blastocysts. Catecholamine receptors of all types can be expressed in mammalian preimplantation embryos, with some species-specific differences, and activation of these receptors can significantly influence preimplantation embryo development. Although the physiological role of biogenic monoamine receptors in early embryonic cells is not fully understood, experimental data indicate their involvement in the regulation of cell proliferation, differentiation and survival under physiological as well as unfavorable or pathological conditions (e.g. during maternal stress).

ACKNOWLEDGEMENTS

This study was supported by the project No. ITMS 26220220204 of the Research & Development Operational Programme funded by the ERDF.

REFERENCES

Amireault, P. & Dubé, F. 2005. Intracellular cAMP and calcium signaling by serotonin in mouse cumulus-oocyte complexes. *Molecular Pharmacology*, 68, 1678–1687.

Babeľová, J., Šefčíková, Z., Čikoš, Š., Špirková, A., Kovaříková, V., Koppel, J., Makarevich, A. V., Chrenek, P. & Fabian, D. 2017. Exposure to neonicotinoid insecticides induces embryotoxicity in mice and rabbits. *Toxicology*, 392, 71–80.

Burkuš, J., Čikoš, Š., Fabian, D., Kubandová, J., Czikková, S. & Koppel J. 2013. Maternal restraint stress negatively influences growth capacity of preimplantation mouse embryos. *General Physiology and Biophysics*, 32(1), 129–137.

Burkuš, J., Kačmarová, M., Kubandová, J., Kokošová, N., Fabianová, K., Fabian, D., Koppel, J. & Čikoš, Š. 2015. Stress exposure during the preimplantation period affects blastocyst lineages and offspring development. *Journal of Reproduction and Development*, 61, 325–331.

Chen, Q., Zhang, Y., Peng, H., Lei, L., Kuang, H., Zhang, L., Ning, L., Cao, Y. & Duan, E. 2011. Transient b2-adrenoceptor activation confers pregnancy loss by disrupting embryo spacing at implantation. *Journal of Biological Chemistry*, 286, 4349–4356.

Čikoš, Š., Veselá, J., Iľková, G., Rehák, P., Czikková, S. & Koppel, J. 2005. Expression of beta adrenergic receptors in mouse oocytes and preimplantation embryos. *Molecular Reproduction and Development*, 71, 145–153.

Čikoš, Š., Rehák, P., Czikková, S., Veselá, J. & Koppel, J. 2007. Expression of adrenergic receptors in mouse preimplantation embryos and ovulated oocytes. *Reproduction*, 133, 1139–1147.

Čikoš, Š., Czikková, S., Chrenek, P., Makarevich, A. V., Burkuš, J., Janštová, Ž., Fabian, D. & Koppel J. 2014. Expression of adrenergic receptors in bovine

and rabbit oocytes and preimplantation embryos. *Reproduction in Domestic Animal*s, 19, 92–100.

Čikoš, Š., Fabian, D., Burkuš, J., Janštová, Ž. & Koppel, J. 2015. Expression of dopamine and adrenergic receptors in mouse embryonic stem cells and preimplantation embryos. *Biologia*, 70, 1263–1271.

Diskin, M. G. & Morfia, D. G. 2008. Embryonic and Early Foetal Losses in Cattle and Other Ruminants. *Reproduction in Domestic Animals*, 43(Suppl.), 260–267.

Fabian, D., Bystriansky, J., Čikoš, Š., Bukovská, A., Burkuš, J. & Koppel, J. 2010. The effect on preimplantation embryo development of non-specific inflammation localized outside the reproductive tract. *Theriogenology*, 74, 1652–60.

Faherty, S., Fitzgerald, A., Keohan, M. & Quinlan, L. R. 2007. Selfrenewal and differentiation of mouse embryonic stem cells as measured by Oct4 expression: the role of the cAMP/PAK pathway. *In Vitro Cellular & Developmental Biology – Animal*, 43, 37–47.

Fischer, B., Chavatte-Palmer, P., Viebahn, C., Navarrete Santos, A. & Duranthon, V. 2012. Rabbit as a reproductive model for human health. *Reproduction*, 144, 1–10.

Fleming, T. P., Wilkins, A., Mears, A., Miller, D. J., Thomas, F., Ghassemifar, M. R., Fesenko, I., Sheth, B., Kwong, W. Y. & Eckert, J. J. 2004. Society for Reproductive Biology Founders' Lecture 2003. The making of an embryo: short-term goals and long-term implications. *Reproduction and Fertility*, 16, 325–337.

Hardy, K. & Spanos, S. 2002. Growth factor expression and function in the human and mouse preimplantation embryo. *Journal of Endocrinology*, 172, 221–236.

Henriquez, S., Tapia, A., Quezada, M., Vargas, M., Cardenas, H., Rios, M., Salvatierra, A. M., Croxatto, H., Orihuela, P., Zegers-Hochschild, F., Munroe, D. J. & Velasquez, L. 2006. Deficient expression of monoamine oxidace A in the endometrium is associated with implantation failure in women participating as recipients in oocyte donation. *Molecular Human Reproduction*, 12, 749–754.

Herlenius, E. & Lagercrantz, H. 2001. Neurotransmitters and neuromodulators during early human development. *Early Human Development*, 65, 21–37.

Humblot, P. 2001. Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. *Theriogenology*, 56, 1417–1433.

Iľková, G., Rehák, P., Veselá, J., Čikoš, Š., Fabian, D., Czikková, S. & Koppel, J. 2004. Serotonin localization and its functional significance during mouse preimplantation embryo development. *Zygote*, 12, 205–213.

Janštová, Ž., Burkuš, J., Kubandová, J., Fabian, D., Koppel, J. & Čikoš, Š. 2017. The effect of maternal stress on blastocyst quality depends on maternal physiological status. *General Physiology and Biophysics*, 36, 53–63.

Khatchadourian, C., Menezo, Y., Gerard, M. & Thibault, C. 1987. Catecholamines within the rabbit oviduct at fertilization time. *Human Reproduction*, 2, 1–5.

Khosla, S., Dean, W., Reik, W. & Feil, R. 2001. Culture of preimplantation embryos and its long-term effects on gene expression and phenotype. *Human Reproduction Update*, 7, 419–427.

Kim, M. O., Na, S. I., Lee, M. Y., Heo, J. S. & Han, H. J. 2008. Epinephrine increases DNA synthesis via ERK1/2s through cAMP, Ca2+/PKC, and PI3K/Akt signaling pathways in mouse embryonic stem cells. *Journal of Cellular Biochemistry*, 104, 1407–1420.

Krauss, G. 2014. *Biochemistry of Signal Transduction and Regulation*, 5th edn. Weinheim, Germany: Wiley-VCH Verlag GmbH, 2014.

Kubandová, J., Cikoš, Š., Burkuš, J., Czikková, S., Koppel, J. & Fabian, D. 2014. Amount of maternal body fat significantly affected the quality of isolated mouse preimplantation embryos and slowed down their development. *Theriogenology*, 81, 187–195.

Kwong, W. Y., Wild, A. E., Roberts, P., Willis, A. C. & Fleming, T. P. 2000. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development*, 127, 4195–4202.

Layden, B. T., Newman, M., Chen, F., Fisher, A. & Lowe, W. L. Jr. 2010. G protein coupled receptors in embryonic stem cells: a role for Gs-α signaling. *PLoS One*, 5, e9105.

Lee, M. Y., Heo, J. S. & Han, H. J. 2006. Dopamine regulates cell cycle regulatory proteins via cAMP, Ca2+/PKC, MAPKs, and NF-κB in mouse embryonic stem cells. *Journal of Cellular Physiology*, 208, 399–406.

Markova, L. N., Sadykova, K. A. & Sakharova, N. I. U. 1990. The effect of biogenic monoamine antagonists on the development of preimplantation mouse embryos cultured *in vitro*. *Zhurnal evoliutsionnoĭ biokhimii i fiziologii*, 26, 726–732.

Nepomnaschy, P. A., Welch, K. B., Mcconnell, D. S., Low, B. S., Strassmann, B. I. & England, B. G. 2006 Cortisol levels and very early pregnancy loss in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 103, p. 3938–3942.

Pendleton, R. G., Rasheed, A., Roychowdhury, R. & Hillman, R. 1998. A new role for catecholamines:

ontogenesis. *Trends in Pharmacological Sciences*, 19, 248–251.

Seeling, T., Čikoš, Š., Grybel, K. J., Janštová, Ž., Pendzialek, S. M., Schindler, M., Špirková, A. & Santos, A. N. 2018. A Diabetic Pregnancy Alters the Expression of Stress-Related Receptors in Gastrulating Rabbit Blastocyst and in the Reproductive Tract. *Reproductive Sciences*, 25, 174–184.

Sun, F., Yang, X. J., Lv, H. Y., Tang, Y. B., An, S. M., Ding, X. P., Li, W. B., Teng, L., Shen, Y., Chen, H. Z. & Zhu L. 2015. β2- Adrenoreceptor-mediated proliferation inhibition of embryonic pluripotent stem cells. *Journal of Cellular Physiology*, 230, 2640–2646.

Tanaka, T. S., Kunath, T., Kimber, W. L., Jaradat, S. A., Stagg, C. A., Usuda, M., Yokota, T., Niwa, H., Rossant, J & Ko, M. S. 2002. Gene expression profiling of embryo-derived stem cells reveals candidate genes associated with pluripotency and lineage specificity. *Genome Research*, 12, 1921–1928.

Tan, X. W., Ji, C. L., Zheng, L. L., Zhang, J., Yuan, H. J., Gong, S., Zhu, J. & Tan, J. H. 2017. Corticotrophin-releasing hormone and corticosterone impair development of preimplantation embryos by inducing oviductal cell apoptosis via activating the Fas system: an *in vitro* study. *Human Reproduction*, 32, 1583–1597.

Tang, F., Barbacioru, C., Bao, S., Lee, C., Nordman, E., Wang, X., Lao, K. & Surani, M. A. 2010. Tracing the derivation of embryonic stem cells from the inner cell mass by single-cell RNASeq analysis. *Cell Stem Cell*, 6, 468–478.

Thouas, G. A., Dominguez, F., Green, M. P., Vilella, F., Simon, C. & Gardner, D. K. 2015. Soluble ligands and their receptors in human embryo development and implantation. *Endocrine Reviews*, 36, 92–130.

Ventura-Juncá, P., Irarrázaval, I., Rolle, A. J., Gutiérrez, J. I., Moreno, R. D. & Santos, M. J. 2015. *In vitro* fertilization (IVF) in mammals: epigenetic and developmental alterations. Scientific and bioethical implications for IVF in humans. *Biological Research*, 48, 68.

Veselá, J., Rehák, P., Mihalik, J., Czikková, S., Pokorný, J. & Koppel, J. 2003. Expression of serotonin receptors in mouse oocytes and preimplantation embryos. *Physiological Resesarch*, 52, 223–228.

Von Borell, E., Dobson, H. & Prunier, A. 2007. Stress, behaviour and reproductive performance in female cattle and pigs. *Hormones and Behaviour*, 52, 130–138.

Way, A. L., Barbato, G. F. & Killian, G. J. 2001: Identification of norepinephrine in bovine oviductal fluid by high performance liquid chromatography. *Life Sciences*, 70, 567–576.

Zhao, X., Ma, W., Das, S. K., Dey, S. & Paria, B. C. 2000. Blastocyst H2 receptor is the target for uterine histamine in implantation in the mouse. *Development*, 127, 2643–2651.

Zheng, L. L., Tan, X. W., Cui, X. Z., Yuan, H. J., Li, H., Jiao, G. Z., Ji, C. L. & Tan, J. H. 2016. Preimplantation maternal stress impairs embryo development by inducing oviductal apoptosis with activation of the Fas system. *Molecular Human Reproduction*, 22, 778–790.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 52, 2019 (1): 30–38

ISSN 1337-9984
E-ISSN 1338-0095

GENETIC VARIABILITY OF HOLSTEIN CATTLE ASSESSED BY PEDIGREE ANALYSIS

Ivan PAVLÍK[1]*, Ondrej KADLEČÍK[2], Ján HUBA[1], Dana PEŠKOVIČOVÁ[1], Martina GONDEKOVÁ[1]

[1]NPPC – Research Institute for Animal Production Nitra, Lužianky, Slovak Republic
[2] Slovak University of Agriculture in Nitra, Department of Animal Genetics and Breeding Biology, Nitra, Slovak Republic

ABSTRACT

The aim of this paper was to evaluate the genetic variability within Holstein cattle population in Slovakia using the methods of pedigree analysis. Totally, 76,176 animals were included into the reference population. Pedigree completeness assessed by index of pedigree completeness was satisfying; the proportion of known ancestors in the first three generations was over 95 %. The value of average inbreeding coefficient was not alarming (0.95 %), but regular increase of average inbreeding was observed in recent years (R^2 = 0.963). The difference between the effective number of founders and the effective number of ancestors showed unequal contributions of individuals into reference population caused by the bottleneck effect. Very low effective numbers of founder genomes reflected the loss of founder gene pool within the population. Regular monitoring of genetic diversity is an essential part of breeding work within the population. Farmers should focus on appropriate mating strategies, e.g. individual mating programs limiting inbreeding.

Key words: founder; gene origin; inbreeding; relatedness

INTRODUCTION

Holstein is considered to be the most common dairy cattle breed world-wide as well as in the Slovak Republic. Nowadays, Holstein represents more than 60 % of dairy cattle population in Slovakia. Slovak Holstein population is open and intensively interacted with the other important Holstein populations (USA, Canada, Germany, France and Netherlands). Genetic variation or diversity could be described and observed as a spectrum of alleles and genotypes (Toro *et al.*, 2011). For the Holstein breed, results obtained in different countries, with various indicators, showed that managing the genetic variability of this world-wide breed deserves much attention (Danchin-Burge *et al.*, 2012). Genetic variation and its maintenance are of the utmost relevance to selection and conservation; therefore, one of the first steps is to estimate the current state and predicted changes in variation. Genealogical information would yield comprehensive parameters to assess the actual levels of diversity, and, therefore should be preferred to assessing the state of variation, although molecular markers, and in the future even whole genome sequences of individuals, are also useful in describing variation (Toro *et al.*, 2011). Knowing the level of genetic diversity within local livestock breeds plays important role in preservation, utilization and production quality in these populations (Kasarda *et al.*, 2015). The breeding strategies currently applied in dairy cattle are very effective in generating genetic gain. However, the reproductive technologies have

***Correspondence:** E-mail: pavlik@vuzv.sk
Ivan Pavlík, NPPC – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic
Tel.: +421 37 6546 595

Received: May 14, 2018
Accepted: January 18, 2019

increased the focus on a small number of superior animals, especially bulls, and the advanced methods of breeding value estimation have increased the accuracy of prediction by using information on all available relatives. Both of these advancements in animal breeding have increased the probability of generating inbred animals (Sørensen *et al.*, 2005). The trend in inbreeding is doubtlessly the tool most frequently used to quantify the rate of genetic drift. Another complementary approach is to analyse the probabilities of gene origin. In this method, the genetic contributions of the founders, i.e. the ancestors with unknown parents, of the current population are measured. This method assesses how an original gene pool has been maintained across generations (Boichard *et al.*, 1997). Pedigree analysis as a tool for diversity evaluation was published by various authors (Baumung and Sölkner, 2002; Bouquet *et al.*, 2011; Danching-Burge *et al.*, 2012; Gutiérrez *et al.*, 2003; Hammami *et al.*, 2007; Maignel *et al.*, 1996). Previously, genealogical analysis in Slovak cattle populations was presented by Kadlečík *et al.* (2013) in three dairy breeds in Slovakia, Kadlečík *et al.* (2011) in Pinzgau population, Kadlečík and Pavlík (2012) in four beef breeds, Hazuchová *et al.* (2012) and Hazuchová *et al.*, (2013) in Simmental cattle; and Pavlík *et al.* (2012) in Holstein cattle and Pavlík *et al.* (2014) in Pinzgau cattle. Intensive selection and using a limited number of superior animals in recent years signify potential risk of genetic diversity loss even within large livestock populations (including Holstein breed). Therefore, the objective of this study was to assess the inbreeding and diversity measures based on the probability of gene origin within the Holstein cattle population in Slovakia by means of pedigree analysis trying to estimate important factors affecting the diversity state.

MATERIAL AND METHODS

Pedigree information was obtained from The Breeding Services of Slovak Republic, s.e. Living cows involved in official animal recording and bulls with reserve of frozen semen doses were taken into account. The reference population was represented by 76,176 animals, of which 75,835 were cows and 341 were bulls. Animals belonging to the reference population with their ancestors represented whole pedigree file (248,474 individuals). Both colour subpopulations of Holstein cattle (Black&White – H and Red&White – R) were considered. The minimal gene proportion of Holstein breed in each individual was 50.0 %. All bulls were considered as purebred (over 93.75 % Holstein gene proportion). In the case of cows, 59.34 % of animals were purebred; the rest of the cow population was represented by various types of crossbred animals (50.0 – 93.74 % Holstein gene proportion). The reference population was divided into groups according to sex and colour subpopulation. The number of animals in the defined subpopulations is presented in Table 1.

Table 1. Number of animals under study

Population	*N*
Pedigree file	248,474
Reference population	76,176
RP – bulls	341
RP – cows	75,835
RP – H bulls	257
RP – R bulls	84
RP – H cows	52,082
RP – R cows	23,753

The first step of our analysis was to assess the pedigree depth as an important factor affecting reliability of genetic variation evaluation. Pedigree depth was evaluated by an index of pedigree completeness (PEC) according to McCluer *et al.* (1983). PEC was calculated according to the following formula:

$$PEC = \frac{2C_{sire}\, C_{dam}}{C_{sire}\, C_{dam}},$$

where C_{sire} and C_{dam} are contributions from the paternal and maternal lines, respectively.

$$C = \frac{1}{d} \sum \frac{d}{i} = {}_1 g_i ,$$

where g_i is the proportion of known ancestors in generation *i*; and *d* is the number of generations that is taken into account.

Inbreeding coefficient (*F*), defined as the probability that an individual has two identical alleles by descent (Malécot, 1948), was computed according to the algorithm of Meuwissen and Luo (1992). Inbreeding trend in the reference population was calculated by means of moving average (two years moving average taken into account).

Genetic variation was evaluated according to measures based on probability of gene origin as well. Following measures based on probability of gene origin were used:

- number of founders (*f*); founder is defined as the animal with unknown genetic connections to other animals in pedigree except its own progeny (Lacy, 1989).
- effective number of founders (f_e), defined as the number of equally contributing founders that will produce the same genetic diversity as in the assessed population (Boichard *et al.*, 1997), was calculated according to this formula:

$$f_e = 1 / \sum_{k=1}^{f} q_k^2,$$

where q_k represents the probability of gene origin of the *k* ancestor.

- effective number of ancestors (f_a) defined as the minimal number of ancestors necessary to explain the genetic diversity in the reference population (Boichard *et al.*, 1997) which was computed as:

$$f_a = 1 / \sum_{j=1}^{a} q_k^2,$$

where p_k is the marginal contribution of ancestor *k*.

- effective number of founder genomes (N_g) defined as the probability that a gene from the founder population has been maintained in the reference population for a given locus if all founders had contributed equally and no alleles had been lost by drift during bottlenecks (Lacy, 1989). Effective number of founder genomes has been computed as:

$$N_g = \frac{1}{2} \sum_{k=1}^{2f} f_k^2,$$

where f_k is the frequency of allele k (Boichard *et al.*, 1997).

The computation of diversity measures, F-statistics and pedigree depth was provided by an ENDOG v. 4.8 software package (Gutiérrez and Goyache, 2005).

RESULTS AND DISCUSSION

Pedigree completeness

Siderits *et al.* (2013) presented that a relatively small improvement of the pedigree information may lead to apparent changes in the measurements of genetic variability. Therefore, deeper pedigrees may offer more reliable information for diversity evaluation. In our case, the pedigree quality was expressed by the index of pedigree completeness (McCluer *et al.*, 1983). As expected, the pedigree completeness was higher in the reference population than in the whole pedigree file. While in the pedigree file, only 69.93 % of animals had both parents known in the first generation, in the reference population, 99.59 % of animals had both parents known. Increasing number of generations taken into account led to decreasing proportion of known ancestors in farther generations. Similar tendency was presented by Sørensen *et al.* (2005) in Danish Holstein population and Hammami *et al.*, (2007) in Holstein populations of Tunisia and Luxembourg. Very similar level of pedigree completeness was observed by Hazuchová *et al.* (2012) and Hazuchová *et al.* (2013) in Slovak Simmental population.

In the case of RP, the ratio of known ancestors in the second generation declined from 95.03 % to 59.24 % in the fifth generation. Significant differences in pedigree completeness were observed between bulls and cows of fifth generation; in farther generations the differences were negligible. The colour variety (H or R) had no impact on the pedigree depth. Pedigree completeness of H and R bulls, as well as cows, was similar with slightly higher tendency in H animals.

Very high pedigree completeness was published by Hagger (2005) in Original Braunvieh cattle in Switzerland, where almost all animals had 100 % completeness of pedigree information in the first five traced generations. Lower level of pedigree depth than in our bulls was presented by Kania-Gierdziewicz (2005) in Polish Holstein sires. Melka *et al.* (2013) found PEC = 97 % in first four traced generations in Canadian Guernsey cattle, while in South African Guernsey cattle it was only 74 %. The quality of pedigrees is different across the countries related to the tradition and history of organized animal breeding. Considerably lower pedigree completeness than in our case

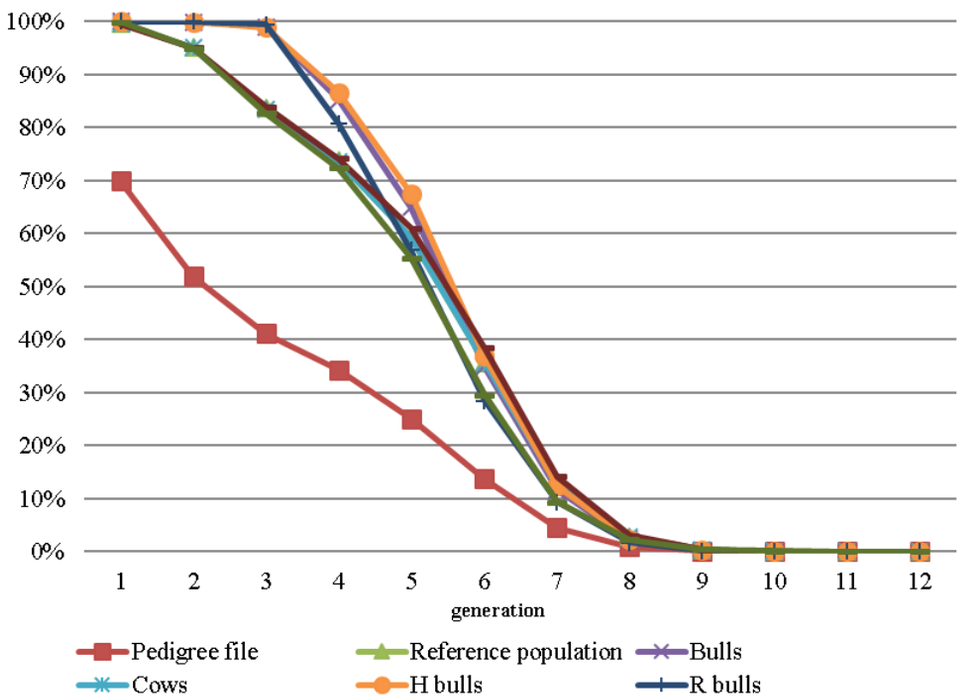


Figure 1. Pedigree completeness in investigated populations

was presented by Malhado *et al.* (2010) in Brazilian Nelore cattle. The overview of pedigree completeness in given populations is presented in Figure 1.

Inbreeding and its evolution

Inbreeding in dairy cattle populations is one of the most important factors affecting the diversity. Many authors confirmed negative effect of inbreeding on different traits (e.g. Maximini *et al.*, 2011; Kasarda and Kadlecik, 2007; Fuerst-Waltl and Fuerst, 2012; Panetto *et al.*, 2010). In addition the average inbreeding value, the trend of inbreeding across the years might be used for managing breeding programs to avoid negative effects of inbreeding depression. In our case, average inbreeding coefficient in the given populations (Table 2) and its evolution across the animals' years of birth (Figure 2) was considered.

The average inbreeding coefficient ranged from 0.34 % in the whole pedigree file to 2.23 % in H bulls. The average inbreeding intensity was 0.95 % in RP. The intensity of inbreeding was more significant in H animals (bulls and cows) than in R animals. This fact was surprising because the total population size of H animals was almost twice as large as in the Red-Holstein animals. Inbreeding intensity of bulls was more significant than in case of cows in both colour subpopulations. The average values of *F* were not alarming but the proportion of inbred animals was very high. The highest proportion of inbred animals was found in H sires (99.22 %) compared to 94.43 % in all bulls, 80.22 in H cows, 79.76 % in R bulls, 73.59 % in RP, 73.50 % in all cows and finally 58.77 % in R cows. The lower inbreeding intensity and proportion of inbred animals in R cows is related to lowest proportion of purebred animals. The highest proportion of various crossbred animals was in R cows. Although the average inbreeding coefficients were not high, farmers should pay attention on preparation of mating plans. Kadlečík *et al.* (2017) presented lower proportion of inbred animals in reference population of the Slovak Simmental cattle (43 %).

Higher inbreeding coefficient than in our study was published by Danchin-Burge *et al.* (2012) in French Holstein population (*F* = 3.80 %). Higher *F* was presented by Kaerney *et al.* (2004) in British

Table 2. Average inbreeding coefficient in investigated populations

Population	Inbreeding coefficient (%)	Standard deviation	Minimum (%)	Maximum (%)
Pedigree file	0.34	1.12	0	38.67
Reference population	0.95	1.71	0	36.25
RP – bulls	1.99	1.65	0	11.91
RP – cows	0.95	1.71	0	36.25
RP – H bulls	2.23	1.64	0	11.91
RP – R bulls	1.25	1.49	0	8.08
RP – H cows	1.13	1.80	0	36.25
RP – R cows	0.54	1.40	0	28.32

Holstein (*F* = 3.06 in bulls, *F* = 2.64 % in cows) and McParland *et al.* (2007) in Irish Holstein (*F* = 1.49 %). Maiwashe *et al.* (2006) presented relatively higher inbreeding intensity in Holstein population of South Africa (*F* = 2.30 %). Very similar inbreeding values as in our study were published by Bouquet *et al.*, (2011) in Charolais populations of Denmark (*F* = 1.04 %), Ireland (*F* = 0.99 %) and Sweden (*F* = 0.92 %). On the other hand, very low inbreeding intensity was found in Brazilian Nelore cattle (*F* = 0.20 %) presented by Malhado *et al.* (2010), but the authors noted that low inbreeding was a result of lower pedigree completeness in the Nelore population. In Slovakia, lower inbreeding intensity was observed by Kadlečík *et al.* (2011) in Pinzgau cattle population (*F* = 0.57 %) and Hazuchová *et al.* (2013) in Simmental cattle (*F* = 0.36 %).

The evolution of inbreeding coefficient through the years showed that in the recent ten years, there was an increase in the mean *F* value of 0.5 % during this period in RP. While animals born in 2002 had average *F* less than 0.6 %, the individuals born ten years later had average *F* = 1.10 %. The tendency of increase of the inbreeding coefficient was significant (R^2 = 0.963). This fact is related to increasing proportion of inbred animals and global increase of co-ancestry between animals. Stachowicz *et al.* (2011) monitored the evolution

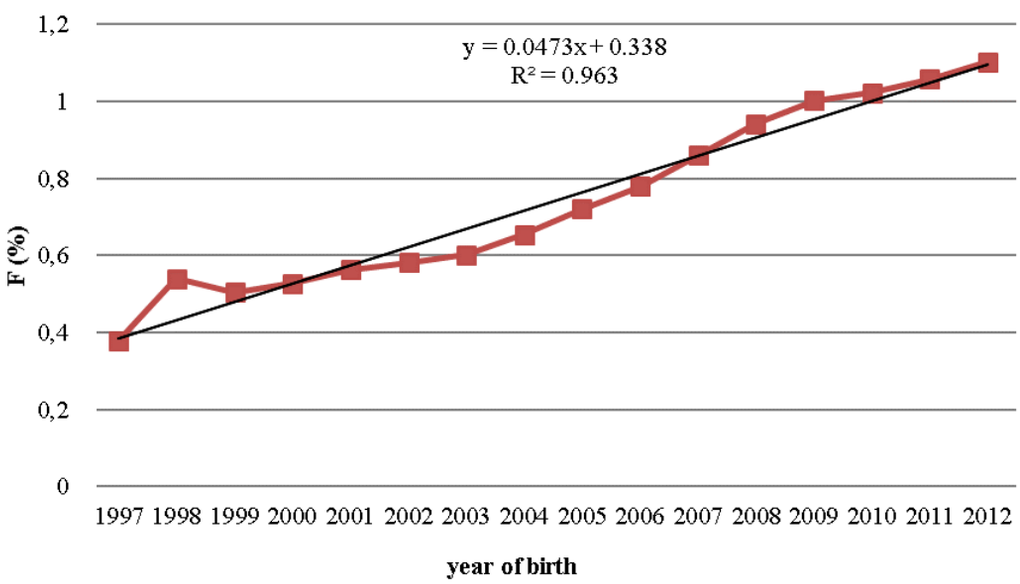


Figure 2. Trend of inbreeding in the reference population

of inbreeding in Holstein population of Canada. They found that animals born in 1968 exceeded the value of 1 % for the very first time. Animals born in 2008 had average *F* over 5 %, which is incomparably higher value than in our population. De Ponte Bouwer *et al*. (2013) observed the highest increase of inbreeding coefficient in the last decade in South African Brown Swiss population. Very similar tendency of *F* increase was presented by Hammami *et al.* (2007) in Holstein populations of Tunisia and Luxembourg. Hazuchová *et al*. (2013) presented significant increase in inbreeding coefficients of Simmental bulls used in the Slovak population born in the recent decade. Similar inbreeding trends were observed by Kadlečík *et al.* (2016) in Slovak populations of beef cattle (Charolais, Blonde d'Aquitaine, Simmental and Limousine).

Probabilities of gene origin

In contrast to the measures based on identity-by-descent (IBD), the characteristics based on gene origin are less susceptible on pedigree quality. Therefore, they represent very useful tool for diversity evaluation. In our case, we focused on number of founders (*f*), effective number of founders (f_e), effective number of ancestors (f_a) and effective number of founder genomes (N_g). In order to assess the influence of bottleneck effect and genetic drift, the f_e/f_a and f_e/N_g ratios were taken into account. The overview of parameters based on probability of gene origin is presented in Table 3.

In our investigation, there was a significant difference between the total number of founders and their effective number in all analysed populations. Presented difference is caused by unequal use of founder gene pool throughout the generations. One of the reasons is the using of limited number of superior sires (placed high in rankings) through artificial insemination. The preference of such mating system is responsible for relatively large amount of offspring per sire in comparison with the other sires and dams. The effective number of founders ranged from 47 in H bulls to 182 in the whole pedigree file. The f_e was 132 in RP, 58 in bulls and 132 in cows. The effective number of ancestors was 133 in the pedigree file, 93 in reference population, 38 in bulls and 93 in cows. In case of R animals, there were higher values of such parameters in comparison with H colour variety. The difference between f_e and f_a values is related to unequal contribution of individual animals into reference population caused by bottleneck effect. The effective number of founder genomes points out the maintenance of founder's gene pool in actual population. The highest N_g value was observed in RP and cows (53 in both cases), while the lowest maintenance of founder gene pool was found in H bulls (N_g = 15). For explaining half of diversity, 105 ancestors sufficed in pedigree file, even 52 ancestors in RP. Only 15 ancestors explained half of diversity in bulls, this number was greater in cows (53). Presented results point out that the loss of founders' variability plays an important role in the overall genetic diversity loss. Danchin-Burge *et al*. (2012) found lower f_e and f_a in French Holstein (f_e = 82; f_a = 21). They presented that the comparison between female and male populations strongly suggests that the impact of AI on the breed is so high that in the long run, the genetic variability of the female population is almost a reflection of the genetic variability of a much smaller population, which is

Table 3. Measures based on probability of gene origin

Population	*f*	f_e	f_a	N_g	f_e/f_a	f_e/N_g	Ancestors explaining 50 % of diversity
Pedigree file	106 953	182	133	-	1.37	-	105
Reference population	106 494	132	93	53	1.42	2.49	52
RP – bulls	664	58	38	19	1.53	3.05	15
RP – cows	106 388	132	93	53	1.42	2.49	53
RP – H bulls	476	47	31	15	1.52	3.13	11
RP – R bulls	304	86	46	21	1.87	4.10	17
RP – H cows	75 932	96	69	37	1.39	2.59	34
RP – R cows	36 490	149	90	49	1.66	3.04	35

considered by the AI bulls. Stachowicz *et al.* (2011) presented higher values of measures based on probability of gene origin in Canadian Holstein females than in males. The same situation was observed in our study. In Canadian Holstein, the effective number of founder genomes was 5.9 in bulls and 7.7 in cows, what indicated massive loss of founders' gene pool in the reference population. The f_e and f_a values of beef cattle breeds were higher than in dairy breeds as presented by Bouquet *et al*. (2011). Similar values of measures based on probability of gene origin were found by Kadlečík *et al*. (2011) in Slovak Pinzgau cattle population (f_e = 141; f_a = 51). Lower values were presented by Hazuchová *et al.* (2013) in the Slovak Simmental cattle.

The ratio of effective number of founders and effective number of ancestors points to the impact of bottleneck effect which reduces the diversity. Greater value of this ratio reflects more significant influence of bottleneck. Presented results showed that the bottleneck reduces the most the diversity in bulls (f_e /f_a = 1.52). Higher impact of bottleneck can be seen in Red-Holsteins (bulls and cows) than in the Black&White variety. The population of bulls was slightly more influenced by the bottleneck than in cows. Differences between the populations were minimal, therefore, it can be concluded that the bottleneck effect has almost the same impact on each population. Bottleneck occurrence in given populations reflects the pedigree construction caused by using small number of superior animals in breeding program. Melka *et al.* (2013) presented that the bottleneck effect reduced the most of the diversity in Canadian Dairy Shorthorn cattle, while the least impact of the bottleneck was observed in Ayrshire cattle. Danchin-Burge *et al.* (2012) found f_e /f_a = 3.90 in French Holstein, what is significantly higher value than in the Slovak Holstein. Moreover, higher impact of the bottleneck effect was found in bulls (f_e /f_a = 4.79). According to the paper of Hazuchová *et al.* (2013), the f_e /N_g ratio in Slovak Simmental cattle was 2.5 times higher than in our study. Therefore, the genetic drift plays more important role in the diversity loss of the Slovak Simmental cattle than in the Slovak Holstein population.

The ratio of effective number of founders and effective number of founder genomes points to the influence of genetic drift on given populations. The highest value was observed in H bulls (f_e /N_g = 3.13), while the lowest one in RP and cows (f_e /N_g = 2.49). Differences between investigated populations were more significant, therefore the impact of genetic drift, defined as a random change in allelic frequencies throughout the generations, differs within the populations. The results obtained from our analysis showed that the bottleneck effect and genetic drift are important factors reducing genetic variability within Holstein cattle population in Slovakia. Significant decline of Holstein cow's number in Slovakia in recent years has led to a significant bottleneck effect occurrence.

CONCLUSION

Despite the fact that the Holstein represents the largest dairy cattle population in Slovakia, it can be considered as strongly influenced by the diversity loss. The limited number of superior sires used in AI led to increase of co-ancestry and inbreeding level, as well as unequal using of the founder gene pool. The disequilibrium between effective number of founders and ancestors and effective number of founder genomes confirmed important impact of the bottleneck effect and genetic drift. Presented results point out the need to include regular diversity monitoring into the breeding program of Holstein cattle in Slovakia. Genetic diversity within the population is necessary to ensure further production use and fitness of the population. Therefore, optimal mating strategy should be found and used to maintain economical sustainability of the Holstein cattle in Slovakia. Using genomic sires is an option how to reduce inbreeding within the population (knowing "real" genomic inbreeding coefficients and "real" genomic relatedness). Therefore, combination of pedigree data and genomic information (including female population) is the optimal strategy for diversity management in the Holstein population of Slovakia.

REFERENCES

Baumung, R. & Sölkner, J. 2002. Analysis of pedigree of Tux-Zillertal, Carinthian Blond and Original Pinzgau cattle population in Austria. *Journal of Animal Breeding and Genetics*, 119, 175–181.

Boichard, D., Maignel, L. & Verrier E. 1997. The value of using probabilities of gene origin to measure genetic variability in a population. *Genetics Selection Evolution*, 29(1), 5–23.

Bouquet, A., Venot, E., Laloë, D., Forabosco, F., Fogh, A., Pabiou, T., Morre, K., Eriksson, J. A., Renand, G. & Phocas, F. 2011. Genetic structure of the European Charolais and Limousin cattle metapopulations using pedigree analyses. *Journal of Animal Science*, 89, 1719–1730.

Danchin-Burge, C., Leroy, G., Brochard, M., Moreaux, S. & Verrier, E. 2012. Evolution of the genetic variability of eight French dairy cattle breeds assessed by pedigree analysis. *Journal of Animal Breeding and Genetics*, 129, 206–217.

De Ponte Bouwer, P., Visser, C. & Mostert, B. E. 2013. Analysis of inbreeding of the South African Dairy Swiss Breed. *South African Journal of Animal Science*, 43(1), 1–6.

Fuerst-Waltl, B. & Fuerst, C. 2012. Effect of inbreeding depression on survival of Austrian Brown Swiss calves and heifers. *Journal of Dairy Science*, 95(10), 6086–6092.

Gutiérrez, J. P., Altarriba, J., Díaz, C., Quintanilla, R., Cañon, J. & Piedrafita, J. 2003. Pedigree analysis of eight Spanish beef cattle breeds. *Genetics Selection Evolution*, 35, 43–63.

Gutiérrez, J. P. & Goyache, F. 2005. A note on ENDOG: a computer program for analysing pedigree information. *Journal of Animal Breeding and Genetics*, 122, 172–176.

Hagger, C. 2005. Estimates of genetic diversity in the brown cattle population of Switzerland obtained from pedigree information. *Journal of Animal Breeding and Genetics*, 122, 405–413.

Hammami, H., Croquet, C., Stoll, J., Rekik, B. & Gengler, N. 2007. Genetic diversity and joint-pedigree analysis of two importing Holstein populations. *Journal of Dairy Science*, 90(7), 3530–3541.

Hazuchová, E., Kadlečík, O., Pavlík, I., Kasarda, R. & Žitný, J. 2012. The assessment of genetic diversity and analysis of pedigree completeness in purebred Slovak Spotted cattle. *Acta agriculturae Slovenica*, 97(3), 131–135.

Hazuchová, E., Kadlečík, O., Pavlík, I. & Kasarda, R. 2013. Assessment of genetic diversity using characteristics based on the probability of gene origin in the Slovak Spotted breed. *Acta fytotechnica et zootechnica*, 16(2), 31–37.

Kadlečík, O., Hazuchová, E., Pavlík, I. & Kasarda, R. 2013. Diversity of cattle breeds in Slovakia. *Slovak Journal of Animal Science*, 46(4), 145–150.

Kadlečík, O., Kasarda, R., Pavlík, I. & Hazuchová, E. 2011. Pedigree Analysis of Slovak Pinzgau Breed. *Agriculturae Conspectus Scientificus*, 76(3), 165–168.

Kadlečík, O. & Pavlík, I. 2012. Genealogical analysis in small populations: The case of four Slovak beef cattle breeds. *Slovak Journal of Animal Science*, 45(4), 111–117.

Kadlečík, O., Pavlík, I., Moravčíková, N. & Kasarda, R. 2016. Inbreeding and genetic diversity loss of four cattle beef breeds in Slovakia. *Acta fytotechnica et zootechnica*, 19(2), 59–63.

Kadlečík, O., Hazuchová, E., Moravčíková, N., Kukučková, V. & Kasarda. R. 2017. Genetic diversity in Slovak Spotted breed. *AGROFOR International Journal*, 2(3), 124–130.

Kasarda, R., Trakovická, A., Moravčíková, N., Šidlová, V. & Kadlečík, O. 2015. Research on diversity, utilization and production quality of local breeds in Slovakia. *Poljoprivreda*, 21(1), 11–15.

Kaerney, J. F., Wall, E., Villanueva, B. & Coffey, M. P. 2004. Inbreeding trends and aplication of optimized selection in the UK Holstein population. *Journal of Dairy Science*, 87(10), 3503–3509.

Kania-Gierdziewicz, J. 2005. Inbreeding and relationship in Polish Black-and-White sires. *Journal of Applied Genetics*, 46(2), 187–193.

Kasarda, R. & Kadlečík, O. 2007. An economic impact of inbreeding in the purebred population of Pinzgau cattle in Slovakia on milk production traits. *Czech Journal of Animal Science*, 52(1), 7–11.

Lacy, R. C. 1989. Analysis of founder representation in pedigrees: founder equivalents and founder genome equivalents. *Zoo Biology*, 111–123.

Maignel, L., Boichard, D. & Verrier, E. 1996. Genetic variability of French dairy breeds estimated from pedigree information. *Interbull Bulltein*, 14, 49–54.

Maiwashe, A., Nephawe, K. A., Weisthuizen, R. R., Mostert, B. E. & Theron, H. E. 2006. Rate of inbreeding and effective population size in four major South African dairy cattle breeds. *South African Journal of Animal Science*, 36 (1), 50–57.

Malécot, G. 1948. *Les Mathématiques de l´Hérédite*. Masson et Cie, Paris, France (in French).

Malhado, C. H., Carneiro, P. L., Malhado, A. C., Filho, R., Bozzi, R. & Ladle, R. J. 2010. Genetic improvement and population structure of the Nelore breed in Northern Brazil. *Pesquisa Agropecuária Brasileira*, 45(10), 1109–1116.

Maximini, L., Fuerst-Waltl, B., Gredler, B. & Baumung, R. 2011. Inbreeding depression on semen quality in Austrian dual-purpose simmental bulls. *Reproduction in Domestic Animals*, 46(1), 102–104.

MacCluer, J. W., Boyce, B., Dyke, L., Weitzkamp, D., Pfenning, A. & Parsons, C. 1983. Inbreeding and pedigree structure in Standardbred horses. *Journal of Heredity*, 74, 394–399.

Mc Parland, S., Kaerney, J. F., Rath, M. & Berry, D. P. 2007. Inbreeding trends and pedigree analysis of Irish dairy and beef cattle populations. *Journal of Animal Science*, 85, 322–331.

Melka, M. G., Stachowicz, K., Miglior, F. & Schenkel, F. S. 2013. Analyses of genetic diversity in five Canadian dairy breeds using pedigree data. *Journal of Animal Breeding and Genetics*, 130, 476–486.

Meuwissen, T. H. E. & Luo Z. 1992. Computing inbreeding coefficients in large populations. *Genetics Selection Evolution*, 24, 305–313.

Panetto, J. C. C., Gutiérrez, J. P., Ferraz, J. B. S. , Cunha, D. G. & Golden B. L. 2010. Assessment of inbreeding depression in Guzerat dairy herds: Effects of individual increase in inbreeding coefficients on production and reproduction. *Journal of Dairy Science*, 93(10), 4902–4912.

Pavlík, I., Hazuchová, E., Kadlečík, O. & Kasarda, R. 2012. *Pedigree analysis of Slovak Holstein cattle*. Book of Abstracts of the 63rd Annual Meeting of the European Federation of Animal Science, Bratislava: Slovakia 27 – 31 August 2012, No. 18, p. 246.

Pavlík, I., Sölkner, J., Kadlečík, O., Kasarda, R., Mészáros, G., Fuerst, Ch. & Fuerst-Waltl, B. 2014. Joint genealogical analysis as a tool for diversity evaluation in Pinzgau cattle populations. *Archiv Tierzucht*, 57(14), 1–12.

Siderits, M., Baumung, R. & Fuerst-Waltl, B. 2013. Pedigree analysis in the German Paint Horse: Genetic variability and the influence of pedigree quality. *Livestock Science*, 151, 152-157.

Sørensen, A. C., Sørensen, M. K. & Berg, P. 2005. Inbreeding in Danish dairy cattle breeds. *Journal of Dairy Science*, 88(5), 1865–1872.

Stachowicz, K., Sargolzaei, M., Miglior, F. & Schenkel, F. S. 2011 – Rates of inbreeding and genetic diversity in Canadian Holstein and Jersey cattle. *Journal of Dairy Science*, 94(10), 5160–5175.

Toro, M. A., Meuwissen, T. H. E., Fernández, J., Shaat, I. & Mäki-Tanila A. 2011. Assessing the genetic diversity in small farm animal populations. *Animal*, 5 (11), 1669–1683.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 52, 2019 (1): 39–46

ISSN 1337-9984
E-ISSN 1338-0095

INTERACTIVE MODEL OF A DAIRY FARM: SHORT COMMUNICATION

Miroslav ZÁHRADNÍK*, Vojtech BRESTENSKÝ, Ján HUBA

NPPC – Research Institute for Animal Production Nitra, Lužianky, Slovak Republic

ABSTRACT

The objective of this paper is to introduce and illustrate the application EkonMOD milk, the decision support tool concept for dairy farm managements. The purpose of this simple web-based application is to assist dairy farms managers to better understand the dynamics of the dairy herd structure and to improve economically sensible decision-making abilities in Slovak conditions. The first module was developed to raise awareness about replacement heifer rearing costs and it can serve as a tool to evaluate specific economic and production parameters of a user specified dairy operation. The module Emissions from dairy farm was developed to allow individual dairy farm managers to calculate the GHG footprint of user-specified dairy operation. The EkonMOD milk tool introduced by the National Agricultural and Food Centre – Research Institute for Animal Production Nitra (NPPC – RIAP) continuously integrates applications previously developed in the sphere of dairy cow husbandry into one platform under the title "Interactive model of a dairy farm". The research and development team run this open access platform for relevant stakeholders to support the environmental and economic performance of dairy farms and to actively seek sound and smart solutions for the inevitable transition to circular economy and well-developed circular agro food systems in the future, with the key role of animal production.

Key words: decision; tool; dairy farm

INTRODUCTION

Integrated information tools will be a major contributor in the realization of a sustainable development, although they are receiving only limited attention in current research generally (Melville, 2010; Korte *et al*., 2012), and especially in agriculture (Aubert *et al*., 2012). Decision support systems (DSS) software packages have mainly been used by farm advisors and other specialists who work with farmers and policymakers (e.g. Nelson *et al*., 2002; Fraisse *et al*., 2015). For farmers, and their advisers, software tools can facilitate effective farm management by recording data efficiently, analysing it, and generating a series of evidence-based recommendations (Rossi *et al*., 2014). Agricultural production decision-making is becoming more complex, due in part to increased competition caused by the globalization of agriculture and the need to adopt more sustainable farming practices (Rogers *et al*., 2004). Keen and Morton (1978) defined decision support systems as computer systems that collect resources and use the ability of a computer to increase quality of decisions by focusing on semi structured problems. The decision support tools typically provide quantitative output and place emphasis on the end user for final problem solving and decision making (Newman *et al*., 2000). Arnott and Pervan (2005) defined DSS as the area of the information systems' discipline that is focused on supporting and improving managerial decision-making. They are designed to help users make more effective decisions by leading them through clear decision stages and presenting the likelihood of various outcomes resulting from different options (Dicks *et al*., 2014;

***Correspondence:** E-mail: zahradnik@vuzv.sk
Miroslav Záhradník, NPPC – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic
Tel.: +421 37 6546 371

Received: September 27, 2018
Accepted: November 20, 2018

Parker, 2004). Sheng and Zhang (2009) defined DSS as human-computer systems that collect information, process information and provide information based on computer systems. Furthermore, a decision support tools can support the decision-makers in an on-going decision situation or it can prepare them to perform better in the future through decision training (Alenljung, 2008). The development of future sustainable agriculture requires acquisition, application and adaptation of knowledge, with the support of appropriate set of IT (Lindblom *et al*., 2014). However, decision makers often argue that there is no easy way to absorb the information available from the scientific research results, so many of the decisions are limited by inadequate or incomplete datasets (Elhag and Walker, 2011). In addition, the farmer needs to develop planning strategies that achieve maximum socio-economic benefits and eco-environmental quality on a macro scale through the optimisation of synthetic systems at the country level (Booty *et al*., 2009). To bridge the gap and to tackle the challenges and complexity of a sustainable development of modern agri production, the farmers need DSS that not only provide current and relevant knowledge, but are also tailored to the farmers' specific needs and plans (Leeuwis, 2004). However, despite their apparent value the uptake of DSS by farmers and advisers in many countries has been limited (Alvarez and Nuthall, 2006; Gent *et al*., 2013; Parker *et al*., 1997). Furthermore, the uptake and levels of acceptance of are low, because scientists do fail to capture the actual needs of the farmers in practice, preferring their own attitude and position on given on-farm issues (e.g. McCown, 2002; 2005; Parker & Sinclair, 2001; Öhlmér, 2001: Öhlmér *et al*., 1998). Additional failure factors are lack of confidence, validity, poor user interface design, low adaptability, and the fear of replacing advisors (e.g. McCown, 2002; Parker & Sinclair, 2001). Following this outcomes, Parker & Sinclair (2001) point out that the single unifying predictor of success or failure of a DSS is the extent to which users are involved and participate in design and development processes.

User involvement is showing to be a critical factor also in the study of Harris & Weistroffer (2009). The importance of involving stakeholders as active participants throughout the whole development process was also highlighted in studies by Jakku and Thorburn, Stewart, *et al*., Valls-Donderis *et al*. and Volk *et al*. One of the identified reason for the failure in implementation is the lack of effective communication between users and developers (Hartwick & Barki, 2001). Van Meensel *et al*. (2012) identified reasons for the low adaption rate arguing that some DSS are too complex, terminology and functions are not adapted and irrelevant to the intended users and their activities, and the often mentioned gap between science and practice within agriculture (Van Meensel *et al*, 2012). The literature review of various DSS analyses emphasised the importance of user-friendliness (McIntosh *et al*., 2008; Nguyen *et al*., 2006; Robinson, 2004; Freebairn, 2002). McIntosh *et al*. (2011) also suggested that the DSS should be designed with "user-friendly interfaces based on elucidating the user's needs and capabilities" and be "adaptable to different types of users, based on their knowledge/expertise". The limited use of scientific results in environmentally driven decisions has been partially sourced in low accessibility to relevant scientific literature (Bayliss *et al*., 2012; Matzek *et al*., 2013; Graham *et al*., 2011; Pullin and Knight, 2015; Young and Van Aarde, 2011).

Number of tools, which assist in the decision process for famers, are already available (Andrew *et al*., 2013; Tamayo *et al*, 2010; Zhong-xiao & Yimit, 2008). In general, dairy farms are deficient in the use of advanced projection frameworks such as simulation and optimization (Bewley *et al*., 2010). An efficient DSS ion support system framework is critical for dairy farming management and decision-making (Meadows *et al*., 2005; Cabrera *et al*., 2006). A basic approach to reduce costs is to shorten the non-productive period of dairy heifers, which can be accomplished by breeding heifers earlier to reduce the age at first calving (AFC); Abeni *et al*., 2000; Daniels, 2010). According to the Result of dairy herd milk recording in Slovak republic, which are annually conducted by the Breeding services of Slovak Republic, the optimal AFC for national conditions supports the previous foreign studies and research papers conclusions. Based on these outcomes it can be stated that reducing AFC in a Slovak Holstein herd had improved the length of productive life. The development of these performance indicators for the period since 2010 till 2017 can be found in Table 1.

Table 1. Development of the length of productive life and lifetime yield of Slovak Holstein cows

year	Age at first calving (days)	Length of productive life (days)
2010	829	827
2011	823	862
2012	815	907
2013	815	908
2014	808	910
2015	799	931
2016	796	930
2017	779	960

Source: Breeding Services of Slovak Republic, state enterprise BS SR, Results of cattle performance recording in Slovakia, (2010-2017)

Furthermore, the recent studies by Zahradnik and Huba (2018) and Zahradnik *et al*. (2018) support these results for Slovak Holstein dairy herds in 2017. The Holstein heifers which first calved at 22 months of age were confirmed to have the highest milk yield per lactation as well as lifetime milk yield per day. However, the highest value of lifetime milk yield was reached by Holstein heifers first calving at 24 months of age. The detailed overview can be seen in Table 2.

Development of the EkonMOD milk tool

This chapter draws heavily on previous works by Zahradnik (2017a, 2017b) and studies by Záhradník and Pokrivčák (2016a, 2016b), Zahradnik *et al*. (2018) describing the rationale of the tool and reflecting on our experiences in developing and delivering the DSS for dairy farmer management in Slovak conditions. Generally, each of the applications under the umbrella of the EkonMOD milk platform is used to evaluate the economic consequences of different on-farm strategies. The introductionary module – Number of heifers needed for replacement was based on several herd specific metrics: culling rate indicator for first lactation cows and for remaining stages of lactations in specified herd, stillbirths rate, dairy cow natality, mortality of calves, selection of calves indicator, ratio of heifers born, heifer selection indicator, culled cows that die before disposal, average age at first calving, Selling price of surplus heifers and culled cows and Cost to raise (purchase deficit) heifers. The application offers a graphical interpretation of these formulas and allows to change input variables in the terms of possible or planned on-farm scenarios. This module was developed to raise awareness about replacement heifer rearing costs and it can serve as a tool to evaluate specific economic and production parameters of a user specified dairy operation.

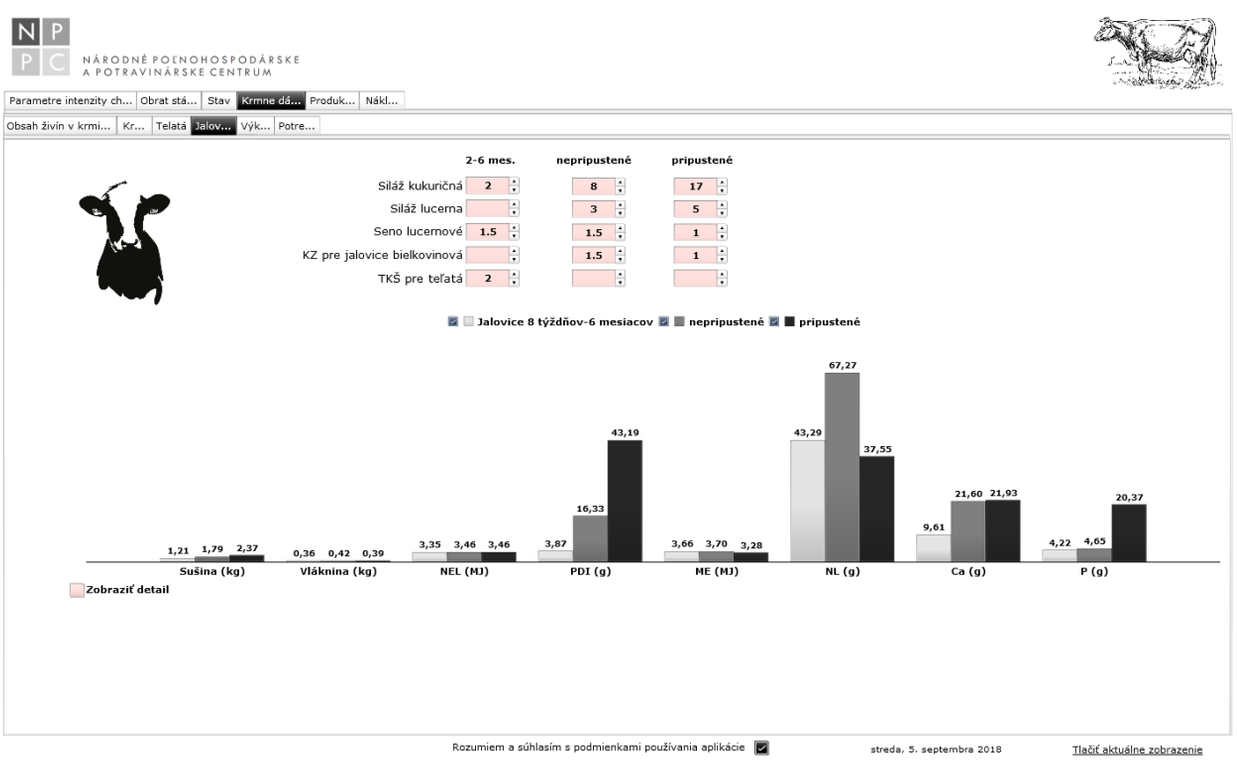
EkonMOD milk tool platform also includes a farm-focused calculator for greenhouse gases (GHG) emissions from a user-specified dairy farm, using following herd specific metrics: annual milk

Table 2. Relation between age at first calving and lifetime yield in Slovak Holstein dairy herds

Age at first calving (days)	Number of lactations	Lifetime yield (kg)	Yield per lactation (kg)	Lifetime yield per day (kg)
22	2,32	21 706	9605	14,46
23	2,44	21 973	9318	13,90
24	2,51	22 514	9064	13,50
26	2,56	22 388	8820	12,66
27	2,59	20 726	8693	11,35
28	2,58	20 863	8534	11,51

yield, number of dairy cows, cow's liveweight (kg), milk fat (%), calving interval, number of cows on pasture, days on pasture, animal waste management system and others. The yields of methane (CH_4), ammonia (NH_3) and nitrous oxide (N_2O) of dairy cows, heifers, calves and fattened bulls, if present on dairy farm. The module Emissions from dairy farm was developed to allow individual dairy farm managers to calculate the GHG footprint of user-specified dairy operation. In addition, the application further determines the emission factors and yields of methane (CH_4), ammonia (NH_3) and nitrous oxide (N_2O) of dairy cows, heifers, calves and fattened bulls, if present on dairy farm.

The NPPC-RIAP research and development team continuously integrates applications developed in the sphere of dairy cow husbandry into one platform under the title "Interactive model of a dairy farm". The application can be found at the address: http://madobis-sk.cvzv.sk/hd/?menu=int_farma. The complex application analyses the input parameters of the breeding intensity, including specified parameters of reproduction and performance, and determines a detailed herd turnover and status of the animals for each category within the given farm. Included in the model is also the determination of the nutritional requirements in feed doses for all categories of animals at the dairy farm. Balancing of the nutritional requirements and the nutritional content of the feed, which the breeder submitted into the model, is done automatically. Following that, the total requirements for feed as well as the storage space and litter requirements. The analysis of total production of marketable products is then, in the context of the particular farm and its characteristics, supplemented by complex evaluation of the milk production costs. The idea behind the application is to not only evaluate the existing state but to provide also an analysis of possible changes, which the farmer is considering or forced to implement. The accuracy, independence and timeliness of business analyses is always based on the relevancy of input parameters, but also on their character, which is that of a business plan. In communicating the idea of individual farm economy evaluation,



Source: Source: EkonMOD milk – Interactive model of a dairy farm screenshot (2018)

Figure 1. Balance between the nutritional and energy content of the feed and heifers requirements

the authors of the application consider this fact to be a decisive influence and therefore neither data nor any other information are archived or otherwise processed. For calculation, the application uses the reproduction and performance parameters input by the user and from this data, it determines herd turnover, status of the animals and nutritional requirements. The user inputs also the feed he plans to feed to the animals and the nutritional content of those. Nutritional requirements per animal category are generated by the application. User defines the portion of each feed in the feed ration and the application determines the difference in nutrient content in the feed ration and the nutritional needs of the animals. By combining the feeds, it is necessary to compose a feed ration in a way that minimises the differences (particularly in dry matter, fibre, Net Energy Lactation (NEL) and Protein Digestible in the Intestine (PDI). An illustrative example of how the application works with a partially unbalanced feeding doses of the considered breeding system is shown on the enclosed screenshot of the application in Figure 1.

We consider perhaps the most important aspect, worth reiterating, to be the application's character of an open platform, which welcomes active participation in the form of feedback and suggestions for further development.

CONCLUSION

Interactive decision support platforms have the potential to address societal concerns related to economic resilient livestock farming system respecting animal welfare standards, lower the environmental burden of production and make resource use more efficient. This paper focused on the development of a range of easy to use tools that promote the implementation of region specific research results with a focus on feeding, reproduction and production of dairy cows. An early warning support system based on farm specific data, primarily derived from user unique inputs, pro-actively alerts the farmer on any economic and production impact of different scenario suggested. The future role of an integrated model of a dairy farm will be to facilitate and connect science and research by delivering more insight into the dynamics of the herd structure and improving the decision making process on the farm level, respecting the needs from practice. The EkonMOD milk tool introduced by the NPPC – RIAP continuously integrates applications previously developed in the sphere of dairy cow husbandry into one platform under the title "Interactive model of a dairy farm". The research and development team run this open access platform for relevant stakeholders to support the environmental and economic performance of dairy farms and to actively seek sound and smart solutions for the inevitable transition to circular economy and well-developed circular agro food systems in the future, with the key role of animal production.

REFERENCES

Abeni, F., Calamari, L., Stefanini, L. & Pirlo, G. 2000. Effects of daily gain in pre- and post pubertal replacement dairy heifers on body condition score, body size, metabolic profile, and future milk production. *Journal of Dairy Science*, 83, 1468–1478.

Alenljung, B. 2008. *Envisioning a future decision support system for requirements engineering: A holistic and human-centred perspective*. Doctoral Thesis, Department of Computer and Information Science, Linköping University, Sweden, Thesis No. 1155.

Alvarez, J. & Nutshall, P., 2006. Adoption of computer based information systems: the case of dairy farmers in Canterbury, NZ, and Florida, Uruguay. *Computers and Electronics in Agriculture*, 50(1), 48–60.

Andrew, M., Grundy, M. & Harris, C. 2013. *Decision support tools for agriculture*, June 4th, 2014. Retrieved from http://www.csiro.au/en/Organisation-Structure/Flagships/Sustainable-Agriculture-Flagship/Decision-support-tools-agri.aspx

Arnott, D. & Pervan, G. 2005. A critical analysis of decision support systems research. *Journal of Information Technology*, 20(2), 67–85.

Aubert, B. A., Schroeder, A. & Grimaudo, J. 2012. IT as enabler of sustainable farming: An empirical analysis of farmers' adoption decision of precision agriculture technology. *Decision Support Systems*, 54, 510–520

Bayliss, H. R., Wilcox, A., Stewart, G. B. & Randall, N. P. (2012) Does research information meet the needs of stakeholders? Exploring evidence selection in the global management of invasive species. *Evidence*

& Policy A Journal of Research Debate and Practice, 8(1), 37–56.

Bewley, J. M., Boehlje, M. D., Gray, A. W., Hogeveen, H., Kenyon, S. J., Eicher, S. D. & Schultz, M. M. 2010. Stochastic simulation using @Risk for dairy businessinvestment decisions. *Agricultural Finance Review*, 70, 97–125.

Booty, W., Wong, I., Lam, D. & Resler, O. 2009. A decision support system for environmental effects monitoring. *Environmental Modelling & Software*, 24, 889–900.

Cabrera, V. E., Hildebrand, P. E., Jones, J. W., Letson, D. & De Vries, A. 2006. An integrated North Florida Dairy Farm model to reduce environmental impacts under seasonal climate variability. *Agriculture Ecosystems and Environment*, 113, 82–97.

Daniels, K. M. 2010. *Dairy heifer mammary development*. Proceedings of 19th Annu. Tri-State Dairy Nutrition Conf., Ft. Wayne, IN. pp. 69–76.

Elhag, M. & Walker, S. 2011. A Decision Support Tool to Assess Desertification Condition in Arid and Semi-Arid Regions. *International Journal of Water Resources and Arid Environments*, 1(5), 378–381.

Fraisse, C. W., Perez, N. & Andreis, J. H. 2015. *Smart Strawberry Advisory System for Mobile Devices*. EDIS Publication AE516, UF/IFAS Extensionhttps://edis.ifas.ufl.edu/pdffiles/AE/AE51600.pdf.

Freebairn, D. M., Robinson, J. B., Glanville, S. F. 2002. *Software tools for learning and decision support*. http://www.apsru.gov.au/apsru/Projects/wfs/pdffiles/ (accessed 6 April 2005).

Gent, D. H., Mahaffee, W. F., Mcroberts, N. & Pfender, W. F. 2013. The use and role of predictive systems in disease management. *Annual Review of Phytopathology*, 51, 267–289.

Graham, R., Mancher, M., Wolman, D. M., Greenfield, S. & Steinberg, E. 2011. *Clinical Practice Guidelines We Can Trust*. National Academic Press.

Harris, M. A. & Weistroffer, H. R. 2009. A New Look at the Relationship between User Involvement in Systems Development and System Success. *Communications of the Association for Information Systems*, 24(42), 739–756.

Hartwick, J. & Barki, H. 2001. Communication as a dimension of user participation. *IEEE Transactions on Professional Communication*, 44(1), 21–36.

Jakku, E. & Thorburn, P. J. 2010. A conceptual framework for guiding the participatory development of agricultural decision support systems. *Decision Support Systems*, 103, 675–682.

Keen, P. G. & Morton, M. S. S. 1978. Decision support systems: An organizational perspective, (35). MA, USA: Addison-Wesley.

Korte, M. & Lee, K. & Fung, C. C. 2012. Sustainability in Information Systems: Requirements and Emerging Technologies. In *Proc. 2012 Int. Conf. on Innovation, Management and Technology Research (ICIMTR 2012)*,Malacca,Malaysia,21-22May,2012,pp.481–485.

Dicks, L. V., Walsh, J. & Sutherland, W. J. 2014. Organising evidence for environmental management decisions: a '4S' hierarchy. *Trends in Ecology & Evolution*, 29(11), 607–613.

Leeuwis, C. (with contributions by A. VAN DEN BAN). 2004. *Communication for Rural Innovation: Rethinking Agricultural Extension*. Oxford: Blackwell Science.

Lindblom, J., Lundström, C. & Ljung, M. 2014. Next generation decision support systems for farmers: sustainable agriculture through sustainable IT. *11th European IFSA Symposium, Farming Systems Facing Global Challenges: Capacities and Strategies*, Proceedings, Berlin, Germany, 1-4 April, pp. 49–57, ref. 30.

Matzek, V., Covino, J., Funk, J. L. & Saunders, M. 2013. Closing the knowing-doing gap in invasive plant management: accessibility and interdisciplinarity of scientific research. *Conservation Letter*s, 7, 208–215.

McCown, R. L. 2005. New Thinking About Farmer Decision Makers. In *The Farmer's Decision: Balancing Economic Successful Agriculture Production with Environmental Quality*. J. L. Hatfield (ed.) Soil and Water Conservation Society, Ankeny, Iowa, USA, pp. 11–44.

McCown, R. L. 2002. Changing systems for supporting farmers' decisions: problems, paradigms, and prospects. *Agricultural Systems*, 74, 179–220.

McIntosh, B. S., Ascough Ii, J. C., Twery, M., Chew, J., Elmahdi, A., Haase, D., Harou, J. J., Hepting, D., Cuddy, S., Jakeman, A. J., Chen, S., Kassahun, A., Lautenbach, S., Matthews, K., Merritt, W., Quinn, N. W. T., Rodriguez-Roda, I., Sieber, S., Stavenga, M., Sulis, A., Ticehurst, J., Volk, M., Wrobel, M.,Van Delden, H., El-Sawah, S., Rizzoli, A. & Voinov, A. 2011. Environmental decision support systems (EDSS) development – Challenges and best practices. *Environmental Modelling & Software*, 26, 1389–1402.

McIntosh, B. S., Giupponi, C., Smith, C., Voinov, A., Assaf, H., Crossman, N., Gaber, N., Groot, J., Haase, D., Hepting, D., Kolkman, R., Matthews, K., Monticino, M.,

Mysiak, J., Quinn, N., Scholten, H. & Sieber, S. 2008. Bridging the gaps between design and use: developing tools to support management and policy. In: Jakeman, A. J., Voinov, A., Rizzoli, A. E. & Chen, S. H. (eds.), *Environmental Modelling, Software and Decision Support: State of the Art and New Perspectives*. Amsterdam: Elsevier.

Melville, N. P. (2010) Information Systems Innovation for Environmental Sustainability. *MIS Quarterly*, 34(1), 1–21.

Nelson, R. A., Holzworth, D. P., Hammer, G. L. & Hayman, P. T. 2002. Infusing the use of seasonal climate forecasting into crop management practice in North East Australia using discussion support software. *Agricultural Systems*, 74(3), 393–414.

Newman, S., Lynch, T. & Plummer, A. A. 2000. Success and failure of decision support systems: learning as we go. *Journal of Animal Science*, 77, 1–12.

Nguyen, N., Wegener, M. & Russell, I. 2006. *Decision support systems in Australian agriculture: state of the art and future development.* Proceedings of the 26th Conference of the International Association of Agricultural Economists, Gold Coast, Australia.

Öhlmér, B. 2001. *Analytic and intuitive decision making – Swedish farmers' behavior in strategic problem solving.* Proceedings of the Third EFITA Conference, Montpellier, France.

Öhlmér, B., Olson, K. & Brehmer, B. 1998. Understanding farmers' decision making processes and improving managerial assistance. *Agricultural Economics*, 18, 273–290.

Parker, C. & Sinclair, M. 2001. User-centred design does make a difference: The case of decision support systems in crop production. *Behaviour & Information Technology*, 20(6), 449-460.

Parker, C., Campion, S. & Kure, H. 1997. *Improving the Uptake of Decision Support Systems in Agriculture.* First European Conference for Information Technology in Agriculture, Citeseer, pp. 129–134.

Parker, C. G. 2004. Decision Support Tools: Barriers to Uptake and Use. *Aspects of Applied Biology*, 72. Advances in Applied Biology: Providing Opportunities for Consumers and Producers in the 21st Century AAB (2004).

Pullin, A. S. & Knight, T. M. 2005. Assessing conservation management's evidence base: a survey of management-plan compilers in the United Kingdom and Australia. *Conservation Biology*, 19, 1989–1996.

Robinson, J. B. 2004. *Understanding and applying decision support systems in Australian farming systems research.* PhD Thesis, University of Western Sydney, NSW, Australia.

Rogers, C. A., Fitzgerald, A. C., Carr, M. A., Covey, B. R., Thomas, J. D. & Looper, M. L. 2004. On-Farm management decisions to improve beef quality of market dairy cows. *Journal of Dairy Science*, 87, 1558–1564.

Rossi, V., Salinari, F., Poni, S., Caffi, T. & Bettati, T. 2014. Addressing the implementation problem in agricultural decision support systems: the example of vite.net®. *Computers and Electronics in Agriculture*, 100, 88–99.

Sheng, Y. & Zhang, S. (2009). Analysis of problems and trends of decision support systems development. Paper presented at *The E-Business and Information System Security (EBISS)*, Wuhan.

Stewart, A., Edwards, D. & Lawrence, A. 2013. Improving the science–policy–practice interface: decision support system uptake and use in the forestry sector in Great Britain. *Scandinavian Journal of Forest Research*. http://dx.doi.org/10.1080/02827581.2013.849358

Tamayo, R. A. C, Ibarra, M. G. L. & Macias, J. A. G. (2010). Better crop management with decision support systems based on wireless sensor networks. Paper presented at *The Seventh International Conference on Electrical Engineering Computing Science and Automatic Control*, Chiapas, Mexico.

Valls-Donderis, P., Duncan, R., Peace, A., Stewart, A., Lawrence, A. & Galiana, F. 2013. Participatory development of decision support systems: which features of the process lead to improved uptake and better outcomes? S*candinavian Journal of Forest Research*. http://dx.doi.org/10.1080/02827581.2013.837950

Van Meensel, J., Lauwers, L., Kempen, I., Dessein, J. & Van Huylenbroeck, G. (2012) Effect of a participatory approach on the successful development of agricultural decision support systems: The case of Pigs2win. *Decision Support Systems*, 54, 164–172.

Volk, M., Lautenbach, S., Van Delden, H., Newham, L. T. & Seppelt, R. 2010. How can we make progress with decision support systems in landscape and river basin management? Lessons learned from a comparative analysis of four different decision support systems. *Environmental Management*, 46, 834–849.

Young, K. D. & Van Aarde, R. J. 2011. Science and elephant management decisions in South Africa. *Biological Conservation*, 144(2), 876–885.

Záhradník, M., Huba, J., Brestenský, V. & Kumičík, M. 2018. EkonMOD milk – interaktívny model farmy dojníc. *AGROmagazín*, 20(5), 30–31.

Záhradník, M. 2017. *Economic optimization of livestock production systems – EkonMOD milk tool*. BIOEAST conference. Ministry of Agriculture, the Research Institute of Agricultural Economics and the National Chamber of Agricultural Economics on 21-22 February, Budapest.

Záhradník, M. 2017. The sustainability of milk production in Slovakia: dissertation thesis / Miroslav Záhradník. – Nitra: SPU v Nitre, 2017. fulltext: http://opac.crzp.sk/?fn=detailBiblioForm&sid=FBA0A0C68B9B2F0CA2CF5BED718F

Záhradník, M. & Huba, J. 2018. Vek jalovíc pri prvom otelení je ekonomicky významným ukazovateľom, jeho optimalizáciu podporuje platforma EkonMOD milk. *Mini info*, 11(4), 23–26.

Záhradník, M. & Pokrivčák, J. 2016. Decision support tool for replacement heifer management: A strategy comparison. In *International Scientific Days 2016: "The Agri-Food Value Chain: Challenges for Natural Resources Management and Society"*: Nitra, SR, 19.-20. 5. 2016: Book of Conference Proceedings. Nitra: SPU v Nitre, 2016, pp. 1002–1008. (Book of Abstracts, p. 146). fulltext: http://spu.fem.uniag.sk/mvd2016/proceedings/en/articles/s12/zahradnik_pokrivcak.pdf

Záhradník, M. & Pokrivčák. J. 2016. Using decision support tool for dairy farm management to assess the economic impact of various feeding scenarios. In *International Scientific Days 2016: "The Agri-Food Value Chain: Challenges for Natural Resources Management and Society"*: Nitra, SR, 19.-20. 5. 2016: Book of Conference Proceedings. Nitra: SPU v Nitre, 2016, pp. 1009–1013. (Book of Abstracts, p. 147). fulltext: http://spu.fem.uniag.sk/mvd2016/proceedings/en/articles/s12/zahradnik_pokrivcak2.pdf

Zhong-Xiao, L. & Yimit, H. 2008. Environmental decision support system development for soil salinization in the arid area oasis. Paper presented at *The International Seminar on Business and Information Management*.

Slovak Journal of Animal Science

http://www.sjas.sk
https://www.sjas.ojs.sk

INSTRUCTIONS FOR AUTHORS

Slovak Journal of Animal Science (SJAS) is an open access (Green Open Access) international peer-reviewed scientific journal published by the National Agricultural and Food Centre – Research Institute for Animal Production Nitra.
Scope: Animal Biotechnology, Animal Husbandry, Animal Nutrition, Animal Product Quality, Animal Environment and Behaviour
Periodicity: 4x per year (March, June, October, December)

Types of Contributions:

- ***Original papers*** – original scientific papers which have not been published yet, should not exceed 8 000 words.
- ***Review articles*** – Contributions of authors with experimental experience and publications on a given topic, focusing on special topics of interest and summarising latest scientific information, up to 10 000 words.
- ***Short communications*** – these may be of a preliminary nature but sufficient for immediate publication, up to 2 500 words.

Manuscript Preparation

The manuscript should be delivered in text editor Microsoft Office (Word, Excel) for Windows. The text should be in non-formatted style (unjustified). It is necessary to number all the lines in the text and pages. Tables and figures (photographs, graphs and other images) should submitted on separate pages appended to the article – approximate place is marked within the text where they should be placed.

Manuscript structure:

Title should briefly but accurately characterize the aim of the paper.
Author(s): Names of authors should be without titles, in capital letters; full first name SURNAME, individual authors separated by a comma.
Abstract should contain abbreviated exact subject of the paper without any added interpretation or criticism. The abstract must not exceed 350 words.
Keywords should contain from 3 to 6 words.
Introduction: brief and clear information about the current state of the research in the given field in our country and abroad (references of authors) and about the objective of the work with explanation of scientific or social necessity to solve it.
Material and Methods should describe the studied material as precisely as possible, as well as used methods of study and ways of evaluation of results.
Results: only concrete results of work, and in text are mentioned also the most important numerical values and data mentioned in tables.
Discussion serves to assess results from various aspects mainly with respect to factors that could influence them, and to confront them with results of other authors.
Results and discussion may comprise of one chapter.
Conclusion has to be clear and brief.
Acknowledgements (If applicable)
References and quotations shall be written according to the **APA style**.

Manuscript Submission

Manuscripts should be submitted using the electronic submission form on the journal's website

https://sjas.ojs.sk/sjas/about/submissions

Unique MS Word file must be saved under the first author's surname only. The author is responsible for originality of the paper, subject-matter and formal attributes of the paper. Papers are published in English language. The author is responsible for translation into English. In case of linguistic shortcomings the paper will be sent back to the author.

The author's declaration
(http://www.vuzv.sk/slju/Declaration.pdf) that the paper has not been published anywhere else should be enclosed.

The Corresponding Author
should include his or her full name including all academic, scientific and pedagogic titles and the detailed address of the institution with postal code, telephone and fax numbers, and e-mail address.

Review Process

All submitted manuscripts will be considered for publication free of charge. All scientific contributions will be peer-reviewed on the criteria of originality and quality by the minimum of two reviewers. Their assessment will be delivered to the author; in case of rejection, the editor makes every effort to explain the causes for it to the author. After the author receives the reviewers' opinion he owes to return the corrected manuscript to the editorial office within three weeks. In an enclosure, the author has to answer all the important comments of the reviewers clearly and in detail. The revised manuscript will be again considered by the original reviewers and the Editorial Board and the final decision will be made.

Full p-to-date instructions for authors are available on the journal website: https://www.sjas.ojs.sk

Slovak Journal of Animal Science, 52, 2019 (1)

Contents

ISSN 1337-9984 (Print)
ISSN 1338-0095 (Online)