Number 3
2020
Volume 53
53 (3) 97–159
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, 95 € 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: sjas@nppc.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, 53, 2020 (3)

Contents

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 53, 2020 (3): 97–103

ISSN 1337-9984
E-ISSN 1338-0095

CRYOPRESERVATION OF RAM SPERM FROM AUTOHTONOUS BREEDS AS A METHOD F0R PRESERVING BIODIVERSITY

Jovana GRBA[1]*, Sava SPIRIDONOVIĆ[1], Saša DRAGIN[1], Barbora KULÍKOVÁ[2], Ivan PIHLER[1], Aleksandar MILOVANOVIĆ[3], Peter CHRENEK[2], Denis KUČEVIĆ[1], Andrej BALÁŽI[2]

[1]University of Novi Sad, Faculty of Agriculture, Republic of Serbia
[2]NPPC – Research Institute for Animal Production Nitra, Lužianky, Slovak Republic
[3]NIV – Scientific Veterinary Institute Novi Sad, Republic of Serbia

ABSTRACT

In the last few decades biodiversity of domestic animals has been declined. The importance of preserving indigenous breeds is reflected in the fact that they contain the original genetic code, are adaptable to adverse conditions and more resistant to disease. In this study we have investigated the possibility of cryopreservation of ram sperm as a method for preserving biodiversity. Cryopreservation of the Wallachian sheep gametes would allow easier exchange of genetic material between geographically distant locations and, thus, expand the genetic diversity of otherwise small and distant locations. The experiment was performed on rams of Wallachian sheep breed. Six semen samples from two rams were collected by electroejaculation. After collection and 60 or 120 min of incubation at 37 °C the sperm was analysed for motility by a computer-assisted sperm analyser. Collected sperm was diluted and frozen in straws using a rapid freezing method. The straws were thawed after one month. Results of this study showed that freezing influenced total and progressive motility compared to fresh sperm (P = 0.000). Comparing progressive motility immediately after thawing, and 60 or 120 min after thawing we found statistically significant differences between both rams (Ram 1, P = 0.0359; Ram 2, P = 0.0361). In this experiment inter-male variability was not confirmed. For further analysis higher number of animals and other breeds need to be tested.

Key words: biodiversity; cryopreservation; ram; sperm

INTRODUCTION

Biodiversity preservation is a process of genetic conservation through renewal of degraded ecosystems and natural habitats and the preservation and recovery of breeds. Sustainable use represents utilization of biodiversity components that does not cause distortion of biodiversity but represents a rational use of natural resources and maintenance of the potential biodiversity (Stanivuk *et al*., 2017).

In the past decades, the number of autochthonous sheep breeds has been declined. The main factors affecting the number of autochthonous sheep breeds are low productivity and unprofitability of their production (Dragin *et al*., 2017). Criteria of breed selection for conservation must be multiple and well and reasonably chosen. Criteria must respect the potential value of the breed that is the genetic constitution and eventually useful genes for future research at breeder discretion. The possibility of losing a breed is also one of the important criteria, because once lost genes or gene combinations can never be brought back in any way (Dragin *et al*., 2015).

***Correspondence:** E-mail: jovana.grba.94@gmail.com
Jovana Grba, University of Novi Sad, Faculty of Agriculture, Republic of Serbia

Received: July 6, 2020
Accepted: July 23, 2020

Animal gene banks play an important role in agricultural production globally for the present and the future and in sustaining the most of production systems and community livelihoods (Kulikova *et al*., 2018). In the last few decades, almost all farm animal breeds are experiencing a significant decrease of genetic diversity (Prentice and Anzar, 2011). Modern biotechnologies in reproduction allowed the production of large number of progeny from a single individual, as well as the use of effective methods of transport and long-term storage of sperm cells, oocytes and early embryos (Patterson and Silversides, 2003).

Gene banks are defined as systematic and organized collection, preservation and exploration of genetic material, by *in situ* (*in vivo*), or *ex situ* (*ex vivo*). The *in situ* method involves preservation of small herd of animal species, breed and lines (Wildt, 1999; Stančić, 1999). *In situ* method is common in rural area where a tradition of breeding autochthonous breeds of domestic animals is present. The disadvantages of *in situ* conservation are brought about by a lack of complete control over the many factors which influence the survival of individuals and therefore the genetic makeup of the conserved population (Henson, 1992; Furukawa *et al*., 1998). The method *ex situ* involves long-term storage of gamets (sperm cells and oocytes) (Johnston and Lacy, 1995; Stančić, 2000; Stančić *et al*., 2001; Stančić *et al*., 2002; Stančić *et al*., 2005; Stanković, 2012) or early embryos by cryopreservation technology (Stančić, 2004; Boettcher *et al*., 2005; Pereira and Marques, 2008; Prentice and Anzar, 2011; Chrenek *et al*., 2013) as well as by cryopreservation of testicular or ovarian tissue somatic cells (Andrabi and Maxwell, 2007; Pereira and Marques, 2008). The major advantage for *ex situ* conservation is the relative cost of collecting, freezing and storage of frozen material, as compared to maintaining large live population (Stančić *et al*., 2013). Improvements in gamete cryopreservation methods may be useful for preserving valuable genetic stock in breeding programs (Anel *et al*., 2006), and also for establishing a semen bank for sheep breeds with an increased risk of loss of genetic variability due to selection programs (Ehling *et al*., 2006; Nel-Themaat *et al*., 2006).

The natural resistance of spermatozoa to cryopreservation varies among species, breeds and even among individual animals of the same breed (Marco-Jiménez and Vincente, 2004). Sperm undergo changes during epididymal maturation and after the addition of seminal fluids (James *et al*., 1999; Martinez-Pastor *et al*., 2006; Yeung *et al*., 2006; Tamayo-Canul *et al*., 2011b), and these changes include variation in the plasma membrane of ram spermatozoa (Hammerstedt and Parks, 1987). These changes in plasma membrane leads to problems with cryopreservation of ram spermatozoa. Ram sperm is more sensitive to freezing than sperm of other species, and therefore, have a low pregnancy rates after cervical AI with frozen-thawed semen (Maxwell *et al*., 1999). Frozen-thawed semen is used for intrauterine insemination, while fresh semen is used for cervical insemination (Maxwell *et al*., 1999). Post-thaw sperm quality is reduced due to the occurrence of cold shock and osmotic stress during the freeze-thawing process (Salamon and Maxwell, 2000). The damage from freezing can be reduced by adding the extenders and cryoprotectants. Common extenders are the egg yolk and soybean lecithin. Optimal cryopreservation of epididymal spermatozoa following accidental death of ram and other endangered species would also markedly help to preserve biodiversity (Kaabi *et al*., 2003; Hishinuma *et al*., 2003; Martinez-Pastor *et al*., 2009; Fernández-Santos *et al*., 2006).

The success of cryopreservation of semen is determined by the rate of sperm dilution. Membrane destabilization and capacitation-like changes in spermatozoa can be linked to excessive dilution, and cryopreservation can have an additive effect on spermatozoa (Akçay *et al*., 2012).

In our study we analysed fresh and frozen-thawed sperm for its quality from two Wallachian rams. The aim was to compare the effect of freezing and different times of incubation on sperm quality.

MATERIAL AND METHODS

Semen collection

Semen from two Wallachian sheep breed rams was collected by electroejaculation. The rectum was cleaned of faeces. A three electrode probes 1" for ram and boar with diameter of 2.54 cm and

length of approximately 16 cm connected to a power source that allowed voltage and amperage control were used (Minitube Electro-ejaculator). The EE regime (automatic mode, type of curve 2 – the power output is linearly increased from 0.5 Volt to 7 Volt) consisted of consecutive series of 2-s pulses of similar voltage, each separated by 2-s break. The initial voltage was 0.5 V, which was increased in each series until maximum of 7 V. Upon reaching a voltage of 7 volts, impulses remained at this level until the ejaculation was complete. After collection, semen was transported to a laboratory in a water bath at 37 °C.

Sperm motility evaluation and cryopreservation

An aliquot taken from each fresh semen sample was used for motility analysis immediately after collection and following 60 or 120 min of incubation at 37 °C. Semen was diluted in a saline (0.9 % NaCl; Braun, Germany) at the ratio of 1:20, immediately placed into a Standard Count Analysis Chamber Leja (depth of 20 microns) (MiniTüb, Tiefenbach, Germany) and evaluated under a Zeiss AxioScope A1 microscope using the CASA system (Sperm VisionTM; MiniTübe, Tiefenbach, Germany). For each sample and repetition, seven microscopic view fields were analysed for average concentration (CON; 1×10^9) and percentage of total motility (TM; motility & gt; 5 $\mu m.s^{-1}$) and progressively moving spermatozoa (PM; motility & gt; 20 $\mu m.s^{-1}$). Rest of the semen samples were used for cryopreservation.

Semen was frozen using a rapid freezing method. Individual semen samples were cooled down to 15 °C for 20 min to minimize cold-shock damage. After cooling, an aliquot of semen was diluted in a commercial diluent (OviXcell, IMV Technologies, France) enriched with 100 mM trehalose (Sigma Aldrich, Germany) to a ratio of 1:1 or 1:2 (v:v). Thereafter, the semen was loaded into 0.25 ml plastic straws and equilibrated at 5 °C for 90 minutes. The straws were suspended horizontally in liquid nitrogen vapours (LNV) 5 cm above the liquid nitrogen (LN_2) level for 10 min (-125 to -130 °C) before being plunged into LN_2 (-196 °C) for storage.

After one month of storage in LN_2, the straws were thawed by immersing into a water bath at 38 °C for 60 s. Sperm motility analysis was done immediately after thawing and after 60 or 120 min of incubation at 37 °C, as stated above.

Statistical analysis

Sperm motility between the two rams and between different times of incubation was compared by a Duncan test using Statistica 13.2 software (TIBCO Software Inc). Values at $P \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Table 1 shows the results of total (TM) and progressive (PM) motility of thawed ram sperm, immediately after thawing. The results in the first table were obtained from the ejaculates from the ram located in the Slovak Republic (Ram no. 1).

From the attached table we can see that in each sample the progressive motility is less than the total motility, and that it decreases with increasing time interval.

Table 1. Motility of thawed sperm at different time intervals, ram 1

Time intervals	TM 00	PM 00	TM 60	PM 60	TM 120	PM 120
Sample 1	12.90 %	4.30 %	17.90 %	12.00 %	15.10 %	7.10 %
Sample 2	13.20 %	7.00 %	17.50 %	10.70 %	13.30 %	7.80 %
Sample 3	16.60 %	9.50 %	18.60 %	10.20 %	16.50 %	10.50 %
Sample 4	19.00 %	9.50 %	17.80 %	7.60 %	15.50 %	5.60 %
Sample 5	17.20 %	9.90 %	19.20 %	12.70 %	18.00 %	8.70 %
Sample 6	16.40 %	6.60 %	14.60 %	8.00 %	6.70 %	3.80 %

TM – Total motility, PM – Progressive motility

The natural resistance of spermatozoa to cryopreservation varies between breeds and even between individuals of the same breed (Marco-Jiménez and Vincente, 2004). Testing of thawed sperm from two rams of the Wallachian breed revealed the motility of the sperm and their suitability for cryopreservation. Cryopreservation process itself is affected by many factors. The composition of cryoprotectants and the rate of dilution before freezing maybe a key factor in sperm freezing (D'Alessandro, 2000).

Table 2 shows the results of total (TM) and progressive (PM) motility of thawed sperm from the ram located in Serbia (Ram no. 2). The total and progressive motility in this ram changed with the increasing time intervals.

Cryopreservation significantly affects the quality of thawed sperm by lowering sperm motility characteristics (Kubovičová *et al*. 2011). The maintenance of sperm function during freezing and thawing depends on several related factors including cooling rate, equilibration and freezing technique (Salamon and Maxwell, 2000; Bailey *et al*. 2000; Curry, 2000; Anel *et al*. 2006).

In Table 3, we compared the total (TM) and progressive (PM) motility of fresh and thawed sperm between two rams.

The results in the Table 3 showed high statistically significant differences between fresh and thawed sperm in both rams. Ram sperm are sensitive to extreme temperature changes during the freezing process (Salamon and Maxwell, 1995), what leads to sperm changes (Watson, 1995).

Based on the obtained results we can see that there are differences in progressive motility between different time intervals. By comparing progressive motility immediately after thawing, 60 or 120 min after thawing, we found statistically significant differences in both rams between 60 and 120 minutes after thawing. At each stage of the freezing cycle, which includes the process of ejaculate collection, dilution, equilibration, and freezing, sperm may lose the ability to fertilize normally (Watson, 1995).

These results show no statistically significant differences between ejaculates after thawing.

Kubovičová *et al*. (2011) investigated the motility of frozen-thawed sperm between two breeds of

Table 2. Motility of thawed sperm at different time intervals, ram 2

Time intervals	TM 00	PM 00	TM 60	PM 60	TM 120	PM 120
Sample 1	13.20 %	6.30 %	13.60 %	6.80 %	14.20 %	6.90 %
Sample 2	15.80 %	8.20 %	12.50 %	8.90 %	14.50 %	8.60 %
Sample 3	12.65 %	7.40 %	15.40 %	10.20 %	12.60 %	5.30 %
Sample 4	18.90 %	9.60 %	18.70 %	8.50 %	16.90 %	8.20 %
Sample 5	17.40 %	4.90 %	18.20 %	9.10 %	8.60 %	7.90 %
Sample 6	15.50 %	8.70 %	16.80 %	12.30 %	15.10 %	5.80 %

TM – Total motility, PM – Progressive motility

Table 3. Comparison between ram 1 and ram 2 of total and progressive motility of thawed sperm at different time intervals

Sperm motility at different time intervals	Frozen				Fresh			
	TM 60	PM 60	TM 120	PM 120	TM 60	PM 60	TM 120	PM 120
Ram 1	17.60 %	12.00 %^{ab}	14.20 %	7.10 %^{ac}	78.20 %	69.90 %^{b}	78.60 %	69.70 %^{c}
Ram 2	15.90 %	10.70 %^{ad}	13.60 %	7.80 %^{ae}	78.00 %	80.20 %^{d}	75.10 %	76.10 %^{e}

TM – Total motility, PM – Progressive motility, ^{a,b,c,d,e} – significant differences ($P < 0.05$) within a row.

sheep and also obtained significant differences. Total and progressive sperm motility after incubation was significantly reduced. Variability in the quality of thawed sperm in males of the same breed was noted (Waterhouse *et al*., 2006; Lavara *et al*., 2013; Sallem *et al*., 2015; Kulíková *et al*., 2017).

Kulíková *et al*. (2018) found no differences in the total and progressive motility of fresh sperm between two tested Wallachian breed rams. The quality of fresh sperm was similar, but thawed sperm showed a difference and thus confirmed various sensitivity to the cryopreservation procedure.

CONCLUSION

The results of this study indicate that freezing significantly affects total and progressive sperm motility. No difference in total and progressive sperm motility was found between the two rams, but significant differences in sperm motility and progressive motility in both rams 60 and 120 min after thawing were observed in comparison with fresh semen. In conclusion, ram sperm is poorly susceptible to freezing, and it is necessary to perform a new research in order to improve the quality of frozen-thawed sperm.

REFERENCES

Akçay, E., Kulaksız, R., Daşkin, A., Çebi, Ç. & Tekin, K. (2012). The effect of different dilution rates on post-thaw quality of ram semen frozen in two different egg-yolk free extenders. *Slovenian Veterinary Research*, 49(2), 97−102.

Anel, L., Alvarez, M., Martinez-Pastor, F., Garcia-Macias, V., Anel, E. & De Paz, P. (2006). Improvement strategies in ovine artificial insemination. *Reproduction in Domestic Animal*s, 41, 30−42.

Andrabi, S. M. H. & Maxwell, W. M. C. (2007). A review on reproductive biotechnologies for conservation of endangered mammalian species. *Animal Reproduction Science*, 99(3-4), 223−243.

Bailey Janice, L., Jean-Francois, B. & Nathaly, C. (2000). Semen cryopreservation in domestic animals: a damaging and capacitating phenomenon. *Journal of Andrology*, 21, 1−7.

Boettcher, P. J., Stella, A., Pizzi, F. & Gandini, G. (2005). The combined use of embryos and semen for cryogenic conservation of mammalian livestock genetic resources. *Genetics Selection Evolution*, 37(6), 657−675.

Chrenek, P., Kubovicova, E. & Makarevich, A. (2017). Current situation in the gene bank of animal genetic resources in Slovakia: a review. *Slovak Journal of Animal Science*, 2017, 50(4), 135−148.

Curry, M. R. (2000). Cryopreservation of semen from domestic livestock. *Reviews of Reproduction*, 5, 46−52.

D'Alessandro, A. G., Martemucci, G., Colonna, M. A. & Bellitti, A. (2001). Post-thaw survival of ram spermatozoa and fertility after insemination as affected by prefreezing sperm concentration and extender composition. *Theriogenology*, 55(5), 1159−1170.

Dragin, S., Trivunović, S., Stančić, I., Jurakić, Ž. & Šaran, M. (2015). Preservation and Conservation of Animal Genetic Resource in the Republic of Serbia. *The 3rd International Scientific Conference* "*Animal Biotechnology*", Nitra, Slovak Republic, Dec. 10th, 2015. In: Slovak Journal of Animal Science, 48(4), 145−194.

Dragin, S., Šaran, M. & Radović, I. (2017). Preservation and Conservation Of Sheep Genetic Resources In Northern Serbia, Vojvodina, (Invited paper). *The 5th International Scientific Conference "Animal Biotechnology"*, Abstracts: Slovak Journal of Animal Science, 50(4), 159−174.

Ehling, C., Rath, D., Struckmann, C., Frenzel, A., Schindler, L. & Niemann, H. (2006). Utilization of frozen-thawed epididymal ram semen to preserve genetic diversity in Scrapie susceptible sheep breeds. *Theriogenology*, 66, 2160−2164.

Fernández-Santos, M. R., Esteso, M. C., Montoro, V., Soler, A. J. & Garde, J. J. (2006). Cryopreservation of Iberian red deer (*Cervus elaphus hispanicus*) epididymal spermatozoa: effects of egg yolk, glycerol and cooling rate. *Theriogenology*, 66, 1931−1942.

Furukawa, T., Nirasawa, K., Satoh, M., Ishii, K. & Hicks, C. (1998). Sustainable production systems for use and conservation of native pig breeds in developing countries. *Proc. 4th Global Conference on Conservation of Domestic Animal Genetic Resources*, August 17-21, Kathmandu, Nepal.

Hammerstedt, R. H. & Parks, J. E. (1987). Changes in sperm surfaces associated with epididymal transit. *Journal of Reproduction and fertility. Supplement*, 34, 133–149.

Henson, E. L. (1992). *In situ* conservation of livestock and poultry. *FAO Animal Health and Health Paper*, 99, 1−112.

Hishinuma, M., Suzuki, K. & Sekine, J. (2003). Recovery and cryopreservation of sika deer (*Cervus nippon*) spermatozoa from epididymides stored at 4 °C. *Theriogenology*, 59, 813−820.

James, P. S., Wolfe, C. A., Ladha, S. & Jones, R. (1999). Lipid diffusion in the plasma membrane of ram and boar spermatozoa during maturation in the epididymis measured by fluorescence recovery after photobleaching. *Molecular Reproduction and Development*, 52, 207−215.

Johnston, A. L. & Lacy, C. R. (1995). Genome resource banking for species conservation: Selection of sperm donors. *Cryobiology*, 32, 68−77.

Kaabi, M., Paz, P., Alvarez, M., Anel, E., Boixo, J. C., Rouissi, H., Herraez, P. & Anel, L. (2003). Effect of epididymis handling conditions on the quality of ram spermatozoa recovered post-mortem. *Theriogenology*, 60, 1249−1259.

Kubovičová, E., Makarevich, A. V., Špaleková, E. & Hegedušová, Z. (2011). Motility and fertilizing ability of frozen-thawed ram sperm from two sheep breeds, *Slovak Journal of Animal Science*, 44(4), 134−139.

Kulíkova, B., Baláži, A., Tóthová, J., Jurčík, R. & Chrenek, P. (2018). Dilution factor affects the ability of ram sperm to survive cryopreservation: short communitation. *Slovak Journal of Animal Science*, 51(1), 41−44.

Lavara, R., David, I., Mocé, E., Baselga, M. & Vicente, J. S. (2013). Environmental and malevariation factors of freezability in rabbit semen. *Theriogenology*, 79, 582−589.

Marco-Jiménez, F. & Vicente, J. (2004). Ability of frozen-thawed ram spermatozoa to bind to vitrified homologous oocytes, *Spanish Journal of Agricultural Research*, 2(1), 73−77.

Martinez-Pastor, F., Martínez, F., García-Macías, V., Esteso, M. C., Anel, E., Fernández-Santos, M. R., Soler, A. J., de Paz, P., Garde, J. & Anel, L. (2006). A pilot study on post-thawing quality of Iberian red deer spermatozoa (epididymal and electroejaculated) depending on glycerol concentration and extender osmolality. *Theriogenology*, 66, 1165−1172.

Martinez-Pastor, F., Martínez, F., Alvarez, M., Maroto-Morales, A., García-Alvarez, O., Soler, A. J ., Garde, J. J., de Paz, P. & Anel, L. (2009). Cryopreservation of Iberian red deer (*Cervus elaphus hispanicus*) spermatozoa obtained by electroejaculation. *Theriogenology*, 71, 628−638.

Maxwell, W. M., Evans, G., Mortimer, S. T., Gillan, L., Gellatly, E. S. & McPhie, C. A. (1999). Normal fertility in ewes after cervical insemination with frozen-thawed spermatozoa supplemented with seminal plasma. *Reproduction, Fertility and Development*, 11, 123−126.

Nel-Themaat, L., Harding, G. D., Chandler, J. E., Chenevert, J. F., Damiani, P., Fernandez, J. M., Humes, P. E., Pope, C. E. & Godke, R. A. (2006). Quality and freezing qualities of first and second ejaculates collected from endangered Gulf Coast Native rams. *Animal Reproduction Science*, 95, 251−261.

Patterson., D. L. & Silversides F. G. (2003). *Farm animal genetic resourceconservation. Why and how?* http://www.cfagrf.com/. Farm Animal Gentetic Resource Conservation Why and How.

Pereira, R. M., Marques, C. C. (2008). Animal oocyte and embryo cryopreservation. *Cell and Tissue Banking*, 9(4), 267−277.

Prentice, J. & Anzar, M. (2011). Cryopreservation of Mammalian Oocyte for Conservation of Animal Genetics. *Recent Advances in Reproductive Technologies*, Article ID 146405.

Salamon, S. & Maxwell, W. M. C. (1995). Frozen storage of ram semen I: processing, freezing, thawingand fertility after cervical insemination. *Animal Reproduction Science*, 37, 185−249.

Sellem, E., Broekhuijse, M. L. W. J., Chevrier, L., Camugli, S., Schmitt, E., Schibler, L. & Koenen, E. P. C. (2015). Use of combinations of in vitro quality assessments to predict fertility of bovine semen. *Theriogenology*, 84(9), 1447−1454.

Stanivuk, J., Dragin, S., Zekić, V., Stančić, I., Radović, I. & Tikvicki, M. (2017). Sustainable Use Of Podolian Cow Genetic Resources, *The 5th International Scientific Conference "Animal Biotechnology"*, Abstracts: Slovak Journal of Animal Science, 50(4), 159−174.

Stančić, B. (1999). Primena nekih biotehnologija reprodukcije svinja u formiranju genetskih resursa. *Savremena Poljoprivreda*, 48(1-2), 29−37.

Stančić, B. (2000). Savremeni principi tehnologije veštačkog osemenjavanja svinja (pregledni referat po pozivu). *3. Simpozijum "Uzgoj i zaštita zdravlja svinja"*. Vršac, 21-23. jun, Zbornik radova, 35−41.

Stančić, B. (2002). Biotechnology in swine reproduction: A review of our investigations. *Bulletin of University of Agricultural Sciences and Veterinary Medicine*, Cluj (Romania), 57, 232−234.

Stančić, B., Pivko, J. & Grafenau, P. (2005). Our results of biotechnological methods application in control of swine reproductive function. *Savremena Poljoprivreda*, 54(1-2), 47−50.

Stančić, B., Pivko, J., Grafenau, P., Oberfranc, M. & Kubovičova, E. (2001). Rezultati naših istraživanja u oblasti biotehnologije reprodukcije svinja (pregled). *1. Kongres veterinara Republike Srpske (sa medjunarodnim učešćem)*, Banja Luka, 28−30. oktobar, 2001. Zbornik radova, 93.

Stančić, B., Stančić, I., Jotanović, S., Šahinović, R. & Dragin, S. (2013). Prikupljanje i konzervacija oocita i embriona sisara za čuvanje životinjskih genetskih resursa *ex situ* – pregled. *Savremena Poljoprivreda*, 62(3-4), 293−300.

Stanković, B. (2012). *Procena uspeha dubokog zamrzavanja na osnovu kvaliteta spermatozoida u nativnoj spermi nerasta i fertilitet intrauterino osemenjenih krmača (doktorska disertacija)*. Univerzitet u Novom Sadu, Poljoprivredni Fakultet.

Salamon, S. & Maxwell, W. M. (2000). Storage of ram semen. *Animal Reproduction Science*, 62, 77−111.

Tamayo-Canul, J., Álvarez, M., Mata-Campuzano, M., Álvarez-Rodríguez, M., de Paz, P., Anel, L. & Martínez-Pastor, F. (2011b). Effect of storage method and extender osmolality in the quality of cryopreserved epididymal ram spermatozoa. *Animal Reproduction Science*, 129, 188−199.

Waterhouse, K. E., Hofmo, P. O., Tverdal, A. & Miller, Jr. R. R. (2006). Within and between breed differences in freezing tolerance and plasma membrane fatty acid composition of boar sperm. *Reproduction*, 131(5), 887−894.

Watson, P. F. (1995). Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing functions. *Reproduction, Fertility and Development*, 7(4), 871−891.

Wildt, W. D. (1999). *Genome resource banking. Reproductive tissue banking*. Academic Press, San Diego, London, 399−440.

Yeung, C. H., Barfield, J. P. & Cooper, T. G. (2006). Physiological volume regulation by spermatozoa. *Molecular and Cellular Endocrinology*, 250, 98−105.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 53, 2020 (3): 104–109

ISSN 1337-9984
E-ISSN 1338-0095

A HERD-LEVEL STUDY OF REPRODUCTION PROCESS AND POST-PREGNANCY PROBLEMS OF HOLSTEIN DAIRY CATTLE IN IRAN

Shahram SHIRMOHAMMADI[1], Akbar TAGHIZADEH[1]*, Khosrow PARSAEIMEHR[2], Monireh DAREZERSHKIPOOR[2], Neda DIVARI[1]

[1]Department of Animal Sciences, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
[2]Department of Animal Sciences, Faculty of Agriculture, University of Urmia, Urmia, Iran

ABSTRACT

The main purpose of this study was to investigate some critical factors affecting the reproduction process and causing the post-pregnancy problems in Holstein dairy cattle herd. One of the critical factors of concerns for herd managers in industrial dairy farms is timed calving, which can result in a greater profit. To achieve this, timed insemination should be done and the post-partum diseases should be controlled as much as possible so that the subsequent pregnancy would not be delayed. Therefore, this study evaluated the reproduction process in one of the largest industrial dairy farms in Yazd province, Iran. A group of 373 cows was monitored since 2005 to 2014. The obtained results indicated that out of the total number of calving, natural calving had the highest number while the dystocia had the lowest. The rate of post-pregnancy problems was raised by the birth of male calves. It was also observed that as the numbers of parity increased, the rate of natural calving increased considerably, whereas the rate of uterine infection decreased. Nevertheless, the ovarian cyst incidence was increased slightly up to the third parity, while it was decreased after the third calving.

Key words: reproduction; pregnancy problems; Holstein cows

INTRODUCTION

One of the most important reasons of losses of dairy cows in Iran is the reproductive problems. Inadequate reproductive performance of dairy cows, one of the factors of reducing both milk production and calving over the year, can occur in the form of either calving intervals or forced removal of dairy cows, or both of them (Ferguson, 2005; Sewalem *et al.*, 2008). One of the purposes of dairy cow breeding is to enhance the yield and economic productivity using most breeding programs based on milk yield traits (McAllister *et al.*, 1990). However, it is not always a cost-effective issue to increase milk production because it can cause some factors such as prolonging the calving intervals, extending the number of non-pregnancy days and the age of calving, which are not economically viable and efficient (Hansen, 2000). Although in the first and second calving, a strict selection to increase milk production may not directly decrease survival but due to increased diseases, reproductive disorders and other factors, involuntary elimination is increased noticeably based on which longevity in calving and the age of the herd are reduced (Hansen, 2000).

Generally, to obtain higher milk production, we should design an appropriate reproductive program (Plate-Church, 2002). As such, some productive (e.g. milk production) and reproductive traits are in a positive correlation (Veerkamp, 1998). Thus, the selection of bulls for milk production will not have a significant impact on the reproductive performance

***Correspondence:** E-mail: ataghius@yahoo.com
Akbar Taghizadeh, Department of Animal Sciences,
Faculty of Agriculture, University of Tabriz, Tabriz, Iran
Tel.: +989144159663

Received: January 14, 2020
Accepted: July 1, 2020

of dairy cows (Brotherstone *et al.*, 2002; Wall *et al.*, 2003). Nevertheless, proper management can prevent reproductive decline, which is in negative relationship with productive traits. Here, it is worthwhile to mention that the interval between calving and pregnancy (open days) mainly depends on some important factors, such as an artificial insemination technique, calving season, herd management, herd size, production level and the number of parity (calving) (Oseni *et al.*, 2003). In high-performance dairy herds, the attempts to raise the performance may cause a negative impact on fertility (Gröhn & Rajala-Schultz, 2000).

The reproductive efficiency of a cow is usually determined by the age of first calving, the number of non-pregnant days, the number of inseminations per pregnancy and the interval between two calvings (Dematawewa & Berger, 1998). According to the mentioned factors, the aim of this study was to investigate some critical factors affecting both the reproduction and pregnancy of dairy cows.

MATERIAL AND METHODS

Dairy herd information

The study was conducted in the Yazd province (Latitude 31° N' and Longitude 54° E), having an average temperature of 30 ± 2 °C, in an industrial dairy farm (1000 cow) with the mean milk production of 32 kg.d^{-1} being fed three times a day with TMR. The cows were artificially inseminated (1.6 ± 0.1 service per cow).

Data collection

The reproductive traits of 373 dairy cows were monitored in this investigation. All data were collected for ten years (from 2005 to 2014). In addition, cows from the first to the fifth parity were used. The following criteria were observed:

- The sex of born calves
- Dystocia
- Ovarian cysts caused by the birth of male or female calves (Ovarian cysts were determined by a veterinary expert using rectal palpation)
- The effect of the calving parity on the type of delivery
- The effect of calving parity on post-partum diseases (uterine infections were determined by a veterinary expert using rectal palpation)

Statistical analysis

SAS (2009) 9.2 version software was used to analyze the collected data. The Chi-square test was used to compare the obtained data.

RESULTS

The effect of calf sex on the type of delivery as well as on the post-partum diseases is shown in Tables 1 and 2.

These results indicated that among the total numbers of calving in this dairy farm, the ratios of born female calves and male calves were 53.62 % and 46.38 %, respectively. From them, the natural calving was represented at the highest percentage, whereas the dystocia showed the lowest percentage.

Table 1. The impact of calf sex on calving type

Sex	Calving rate (%)	Number of calving	Natural calving (%)	Auxiliary calving (%)	Dystocia (%)	Chi-Square
Bull calf	46.38	173	21.72	17.69	6.97	0.0250
Cow-calf	53.62	200	32.44	16.09	5.09	0.0250
The total of bull and cow-calves	100	373	54.16	33.78	12.06	0.0250

Chi-Square test

Table 2. The impact of calf sex on post-partum diseases (by percentage)

Sex	Ovarian cyst	Uterine infection	Chi-Square
Bull calf	6.90	48.28	0.9366
Cow-calf	5.75	39.08	0.9366
The total of bull and cow-calves	12.64	87.35	0.9366

Chi-Square test

Table 3. The impact of the number of parities on the type of calving (in percentage)

Parity No.	Natural calving	Auxiliary calving	Dystocia	Chi-Square
Parity 1	5.61	8.82	6.15	0.0001
Parity 2	9.89	8.56	2.67	0.0001
Parity 3	13.64	6.95	0.53	0.0001
Parity 4	12.57	6.15	1.07	0.0001
Parity 5	12.57	3.21	1.60	0.0001

Chi-Square test

From the total number of cows with the post-partum disease, 12.64 % had ovarian cysts, while 87.35 % had cervical infection. As the number of parities increased, the rate of natural calving increased as well (Table 3).

At the third parity most of the calvings were natural but after the third parity it decreased slightly. Assisted calving reached its lowest level at the fifth parity. In addition, as the number of parities increased, the dystocia was decreased, but it increased slightly after the third parity. This increase was lower at the fourth and fifth parities as compared to the first and second parities. Table 4 demonstrates the association of the number of parities with the incidence of post-partum diseases.

The ratio of ovarian cysts was notably increased by increasing the number of parities. However, this increase was evident up to the third parity and decreased thereafter. In addition, as the parities progressed, the rate of uterine infection also increased; however, it decreased after the second parity.

Table 4. The impact of the number of calving parity on the post-partum disease (in percentage)

Parity No.	Uterine infection	Ovarian cyst	Chi-Square
Parity 1	2.20	18.68	0.1800
Parity 2	4.40	27.47	0.1800
Parity 3	5.49	20.88	0.1800
Parity 4	0	10.99	0.1800
Parity 5	1.10	8.79	0.1800

Chi-Square test

DISCUSSION

Regarding the experimental results, it was found that the natural calving represented the highest percentage, whereas the dystocia represented the lowest percentage among the calvings. From the total number of cows suffering from post-partum diseases, 12.64 % were related to the ovarian cysts, while about 87.35 % were related to the uterine infection. Our investigations revealed that there was a positive relationship between the calving parity and time of first ovulation after calving. The first and second parities were longer than the first ovulation after calving as compared to the third and more parties. This can be probably due to the lack of energy and high nutritional stress for growth, in addition to lactation needs (Ferguson, 2005). However, it should be noted that there were also high-yielding cows with good reproductive performance (Sewalem *et al.*, 2010), which might be a result of better nutrition and more intensive care. On the other hand, those cows that were genetically determined for more production, although were not delayed in ovulation, but their nutritional deficiencies may have caused some ovarian problems (Dechow *et al.*, 2004).

Based on Table 1, it was concluded that the dystocia in the calving of male calves was considerably higher than that of female calves. By comparing the sexes of calves it was found that with the birth of male calves, the rate of the ovarian cysts and uterine infections became higher than before. Erb *et al.* (1985) reported that dystocia could cause some metritis and pregnancy problems and even lead to the elimination of livestock from the herd.

Some studies indicated that calf size was an essential factor in the type of calving. This is especially important for bull-calve calving. In terms of size, the bull-calves are larger than the cow-calves, which is one of the most important causes of dystocia (Hansen, 2000). Even calf births are the factor in dystocia (Correa *et al.*, 1993). A study conducted by Atashi and Asaadi (2019) showed a direct relationship between calf weight and pregnancy length. It was also noted that in the primiparous cows with a short gestation, the rate of dystocia was higher, it decreased and was found in multiparous cows with average length of gestation. Hammoud *et al.* (2010) stated that dystocia could cause some post-pregnancy diseases as well as some fertility problems. On the other hand, our results showed that as the number of parity increased, the rate of natural calving increased as well, while in the third parity, most of the calvings were natural. Here, it should be noted that the dystocia rate has also decreased by increasing the number of calving parity (Noakes *et al.*, 2009). However, one of the most critical factors in the type of delivery is the age and the body condition score of heifers at the time of insemination. It is worthwhile to mention that heifers should be timely inseminated, as late or early insemination may lead to pregnancy problems as well as to dystocia (Plate-Church, 2002).

It is known that the negative energy balances in high-yielding cows may decrease the reproductive performance as well as the livestock health, especially in first parity cows during early lactation (Wall *et al.*, 2003). This point is especially important in high-yielding cows (Lucy, 2001; Sewalem *et al.*, 2010; Stevenson *et al.*, 1999). The average age at first calving, which is an important indicator to determine the reproductive capacity in heifers and herd capacity to begin production, is between 24 and 25 months (Hare *et al.*, 2006). Faraji-Arough *et al.* (2011) reported that the most appropriate calving age for Holstein cows in Iran was 26.6 months (811.1 days).

The recent review (Noakes *et al.*, 2009) showed that age at first calving is influenced by some environmental and management factors, including proper breeding and nutrition in the pre-calving period. It would be appropriate if the age of first calving does not lead to creating such reproductive problems (Noakes *et al.*, 2009). In this matter, both ovarian cysts and uterine infection have increased significantly by increasing the number of parity. This is due to a relationship between the genetic association suffering from ovarian cysts and milk production. High-yielding cows become more susceptible to suffer from this problem in the first months after calving (Hansen, 2000; Lucy, 2001; Sewalem *et al.*, 2010). On the other hand, one of the major challenges in the reproductive problems in dairy cows are their age and body condition score during insemination (Plate-Church, 2002). Due to the lack of coordination between energy requirements and received energy, the high milk production rate may lead to the generation of a negative

energy balance in cows, which subsequently occurs at the beginning of lactation, when the weight losses and the body condition score is reduced (Lucy, 2000, 2001). Moreover, the mean age of first calving has an important impact on the reproduction capacity of heifers as well as on the capacity of the herd to begin production (Dechow *et al.*, 2004; Hare *et al.*, 2006).

Some researchers showed that cows that are genetically determined for further performance, although have no delay in ovulation, may be late in their first estrus after calving (Dechow *et al.*, 2004). In general, the low heritability and reproductive traits reveal that their variation is more dependent on non-genetic, i.e. environmental and management factors, such as nutrition and diagnostic management (Royal *et al.*, 2002). Some investigations showed that poor nutrition is a critical factor in weight loss that may cause some reproductive losses due to the lack of ovulation. Both overfeeding and obesity may cause a negative effect on reproduction by damaging the folliculogenesis and reducing the oocyte quality (Ferguson, 2005).

CONCLUSION

Of all calvings, natural calving had the highest number whereas the dystocia had the lowest. The rate of post-pregnancy problems was raised by the birth of male calves. It was also observed that as the numbers of parity increased, the rate of natural calving increased considerably, whereas the rate of uterine infections decreased. Nevertheless, the ovarian cyst incidence was increased slightly up to the third parity, while it was decreased after the third calving.

REFERENCES

Atashi, H., & Asaadi, A. (2019). Association between gestation length and lactation performance, lactation curve, calf birth weight and dystocia in Holstein dairy cows in Iran. *Animal Reproduction*, 16(4), 846–852.

Brotherstone, S., Banos, G., & Coffey, M. (2002). Evaluation of yield traits for the development of a UK fertility index for dairy cattle. *Proceedings of the 7th World Congress on Genetics Applied to Livestock Production*, August 19-23, Montpellier, France.

Correa, M., Erb, H., & Scarlett, J. (1993). Path analysis for seven postpartum disorders of Holstein cows. *Journal of Dairy Science*, 76(5), 1305−1312.

Dechow, C. D., Rogers, G., Klei, L., Lawlor, T., & VanRaden, P. (2004). Body condition scores and dairy form evaluations as indicators of days open in US Holsteins. *Journal of Dairy Science*, 87(10), 3534−3541.

Dematawewa, C., & Berger, P. (1998). Genetic and phenotypic parameters for 305-day yield, fertility, and survival in Holsteins. *Journal of Dairy Science*, 81(10), 2700−2709.

Erb, H., Smith, R., Oltenacu, P., Guard, C., Hillman, R., Powers, P., Smith, M., & White, M. (1985). Path model of reproductive disorders and performance, milk fever, mastitis, milk yield, and culling in Holstein cows. *Journal of Dairy Science*, 68(12), 3337−3349.

Faraji-Arough, H., Aslaminejad, A., & Farhangfar, H. (2011). Estimation of genetic parameters and trends for age at first calving and calving interval in Iranian Holstein cows. *Journal of Research in Agricultural Science*, 7(1), 79−87.

Ferguson, J. D. (2005). Nutrition and reproduction in dairy herds. *Veterinary Clinics: Food Animal Practice*, 21(2), 325−347.

Gröhn, Y., & Rajala-Schultz, P. (2000). Epidemiology of reproductive performance in dairy cows. *Animal Reproduction Science*, 60, 605−614.

Hammoud, M., El-Zarkouny, S., & Oudah, E. (2010). Effect of sire, age at first calving, season and year of calving and parity on reproductive performance of Friesian cows under semiarid conditions in Egypt. *Archiva Zootechnica*, 13(1), 60.

Hansen, L. B. (2000). Consequences of selection for milk yield from a geneticist's viewpoint. *Journal of Dairy Science*, 83(5), 1145−1150.

Hare, E., Norman, H., & Wright, J. (2006). Trends in calving ages and calving intervals for dairy cattle breeds in the United States. *Journal of Dairy Science*, 89(1), 365−370.

Lucy, M. (2000). Regulation of ovarian follicular growth by somatotropin and insulin-like growth factors in cattle. *Journal of Dairy Science*, 83(7), 1635−1647.

Lucy, M. (2001). Reproductive loss in high-producing dairy cattle: where will it end? Journal of Dairy Science, 84(6), 1277−1293.

McAllister, A., Vesely, J., Batra, T., Lee, A., Lin, C., Roy, G., Wauthy, J., Winter, K., & McClelland, L. (1990). Genetic changes in protein, milk, and fat yields as a response to selection for protein yield in a closed population of Holsteins. *Journal of Dairy Science*, 73(6), 1593−1602.

Noakes, D., Parkinson, T., & England, G. (2009). Dystocia and other disorders associated with parturition. *Veterinary Reproduction and Obstetrics*, 9, 207−305.

Oseni, S., Misztal, I., Tsuruta, S., & Rekaya, R. (2003). Seasonality of days open in US Holsteins. *Journal of Dairy Science*, 86(11), 3718−3725.

Plate-Church, A. (2002). *Determining Optimal Age at First Calving*. http://www.crigenetica.com.br/upload/artigos/leite/facilidade_parto_novilhas/determining_optimal_age_at_first_calving.pdf

Royal, M., Flint, A., & Woolliams, J. (2002). Genetic and phenotypic relationships among endocrine and traditional fertility traits and production traits in Holstein-Friesian dairy cows. *Journal of Dairy Science*, 85(4), 958−967.

Sas. (2009). SAS/STAT 9.2 User's Guide: The FREQ Procedure, (book Excerpt). SAS Institute Incorporated.

Sewalem, A., Kistemaker, G., & Miglior, F. (2010). Relationship between female fertility and production traits in Canadian Holsteins. *Journal of Dairy Science*, 93(9), 4427−4434.

Sewalem, A., Miglior, F., Kistemaker, G., Sullivan, P., & Van Doormaal, B. (2008). Relationship between reproduction traits and functional longevity in Canadian dairy cattle. *Journal of Dairy Science*, 91(4), 1660−1668.

Stevenson, M., Williamson, N., & Hardon, D. (1999). The effects of calcium supplementation of dairy cattle after calving on milk, milk fat and protein production, and fertility. *New Zealand Veterinary Journal*, 47(2), 53−60.

Veerkamp, R. (1998). Selection for economic efficiency of dairy cattle using information on live weight and feed intake: a review. *Journal of Dairy Science*, 81(4), 1109−1119.

Wall, E., Brotherstone, S., Woolliams, J., Banos, G., & Coffey, M. (2003). Genetic evaluation of fertility using direct and correlated traits. *Journal of Dairy Science*, 86(12), 4093−4102.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 53, 2020 (3): 110–121

ISSN 1337-9984
E-ISSN 1338-0095

DIETARY VITAMIN-MINERAL PREMIX REPLACEMENT WITH LEAF MEAL COMPOSITES IMPROVED THE GROWTH PERFORMANCE OF BROILER CHICKEN

Olugbenga Adeniran OGUNWOLE[1]*, Aderonke Olufunmilola MOSURO[2]

[1]Agricultural Biochemistry & Nutrition Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria
[2]Human Nutrition and Dietetics Department, Faculty of Basic Medical Sciences, Lead City University, Ibadan, Nigeria

ABSTRACT

This study was aimed at evaluating the effects of full or partial replacement of dietary vitamin-mineral premix (VmP) with leaf meal composites (LMC) on performance of broiler chickens. In a completely randomised design, one-day old Arbor Acres plus chicks (n = 936) were randomly allotted to thirteen treatments, each replicated eight times. A replicate comprised nine chicks each. Air-dried leaves of *Telfaria occidentalis*, *Celosia argentea*, *Vernonia amygdalina* and *Moringa oleifera* were milled and constituted in equal proportions to a leaf meal composite (LMC). Diets were formulated with the LMC incorporated in the diets at 1.5, 3.0 and 4.5 % and the VMP at 0.000, 0.125, 0.250 and 0.375 % in a (3 x 4) +1 augmented factorial arrangement. Treatments 1 (T_1), 2 (T_2) and 3 (T_3) contained only 1.5, 3.0 and 4.5 % LMC, respectively, without VmP inclusion. Treatments 4 (T_4), 5 (T_5) and 6 (T_6) each contained 0.125 % VmP alongside 1.5, 3.0 and 4.5 % LMC, respectively. Treatments 7 (T_7), 8 (T_8), and 9 (T_9) also had 0.25 % VmP each alongside 1.5, 3.0 and 4.5 % LMC, respectively. Treatments 10 (T_{10}), 11 (T_{11}) and 12 (T_{12}) each contained 0.375 % VmP with 1.5, 3.0 and 4.5 % LMC, respectively, while Treatment 13 (T_{13}) contained 0.25 % VmP only. Chicks were fed from day 1-21 (starter) and 23-42 (finisher). Increasing dietary VMP supplement resulted in reduced ($p < 0.05$) weight gain (WG) and increased feed conversion ratio (FCR), while higher supplemental LMC increased WG but reduced FCR of chicken. Similar result trends were obtained for the chickens at the finisher phase. Effects of LMC combinations with VmP in chickens on T_2 (2.34 ± 0.22), T_3 (2.33 ± 0.17), T_5 (2.30 ± 0.28) and T_9 (2.00 ± 0.19) on FCR were similar ($p > 0.05$) but lower ($p < 0.05$) than in other treatments. Optimal FCR of 2.25 (starter) and 2.44 (finisher) were attained with 3.43 ($R^2 = 0.73$) and 3.06 % ($R^2 = 0.99$) LMC inclusions, respectively. Thus, LMC successfully replaced VmP in broiler chicken diets without any negative implication on growth performance.

Key words: broiler starter; chicken performance; supplemental vitamin premix; dietary leaf meal; feed conversion ratio

INTRODUCTION

Vitamin-mineral premix (VmP) remained an essential component of feed for broiler chicken as the gut of broilers cannot synthesize adequate vitamin and minerals (Islam *et al*., 2004). Synthetic single vitamin and minerals for producing VmP are manufactured by few companies globally. They are, therefore, expensive and subject to sporadic scarcity, which makes it imperative to seek for viable alternatives to address economic challenges faced by farmers in the rural areas of the developing world. The consistent challenge of importation and scarcity of key ingredients also allows loop of variation in quality and claims by manufacturer of vitamin-mineral premix and the means of proving the veracity of products claim are also lacking (Asaduzzman *et al*., 2005, Ogunwole *et al*., 2012).

***Correspondence:** E-mail: oluogunwole@yahoo.com, oa.ogunwole@ui.edu.ng
Olugbenga Adeniran Ogunwole, Agricultural Biochemistry & Nutrition Unit, Department of Animal Science, University of Ibadan, 20285, Ibadan, Nigeria
Tel: 234 8033939829

Received: January 13, 2020
Accepted: March 17, 2020

Green leafy vegetables have been previously investigated as alternative feed ingredient in form of leaf meal to address economic challenge of high cost of conventional VmP (Tesfaye *et al*., 2013). Leaf meal has been used largely in broiler diets to replace macro feed ingredients like energy and protein (Onyimonyi and Onu, 2009; Gadzirayi *et al*., 2012; Abu *et al*., 2015). Apart from being rich in macro nutrients, such as protein and energy, some green leafy vegetables are known to be innately rich in vitamins and minerals (Rickman *et al*., 2007, Achikanu *et al*., 2013, Uraku *et al*., 2015). The need to find alternative sources of VmP, therefore, places a feasible option on the use of vegetable leaves as ingredient in this category.

The high moisture content of vegetable leaves is, however, a challenge to their use in poultry feed. Different drying methods, employed to retain the inherent nutrients, have been documented (Mosuro, 2018; Mosuro and Ogunwole, 2019). Air drying was the acclaimed method of drying the vegetable leaves (Onayemi and Badifu, 1987; Lakshmi and Vimala, 2000; Krokida and Maroulis, 2007; Sagar and Suresh, 2010; Naikwade, 2014; Mosuro and Ogunwole, 2019).

The VmP developed from locally sourced materials have been successfully used in poultry production in Nigeria (Bolu and Balogun, 2003; Malik *et al*., 2010; Oyewole *et al*., 2013). However, the ingredients employed in the trials were not solely derived from leaf meals. Information on the use of supplemental VmP developed solely from leaf meal in broiler chicken production is still very scanty. Hence, this study was aimed at assessing the effect of replacing dietary VmP supplement with LMC on the growth performance of broiler chicken.

MATERIAL AND METHODS

Experimental location

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The study area lies between longitude of 7°27.05 north and 3°53.74 of the Greenwich Meridian east at an altitude of 200 m above sea level. Average temperature and relative humidity of the location is between 23-42 °C and 60-80 %, respectively (SMUI, 2018). The trial was undertaken between the month of September and November.

Experimental animals and diets

One-day old Arbor Acres plus broiler chicks (n = 936) were randomly allotted to 13 dietary treatments, each treatment was replicated eight times, a replicate comprised nine chicks. The allocation was done using the Experimental Animal Allotment Program (EAAP) version 1.1 according to Kim and Lindemann (2007). A conventional open-sided deep litter house partitioned into 104 cubicles of one square metre each was used to house the birds. Leaves of *Telfaria occidentalis* (HOOK. f),

Table 1. Layout of experimental diets

Dietary Treatment	LMC Inclusion (%)	VmP Inclusion (%)
Treatment 1	1.5	0
Treatment 2	3.0	0
Treatment 3	4.5	0
Treatment 4	1.5	0.125
Treatment 5	3.0	0.125
Treatment 6	4.5	0.125
Treatment 7	1.5	0.25
Treatment 8	3.0	0.25
Treatment 9	4.5	0.25
Treatment 10	1.5	0.375
Treatment 11	3.0	0.375
Treatment 12	4.5	0.375
Treatment 13	0	0.25

Celosia argentea (LINN), *Moringa oleifera* (LAM) and *Vernonia amygdalina* (DEL) were collected, destalked and washed before indoor air-drying on perforated plastic trays to constant weight. The air-dried leaves were milled and mixed in equal proportion to constitute the leaf meal composite (LMC). Detailed description of air drying of the different leaves and constitution into LMC has been succinctly documented (Mosuro and Ogunwole, 2019). The commercial VmP used in the trial was procured from a popular retail outlet in Ibadan, Nigeria.

The thirteen isocaloric and isonitrogenous experimental diets formulated contained LMC or VmP as well as the different combinations of both the LMC and VmP.

The gross composition of the basal starter and finisher diets is shown in Table 2. The chickens were fed the diets and supplied water *ad libitum*. The experiment was a {1+ (3×4)} augmented factorial arrangement in a completely randomised design. The factorial arrangement had three levels of LMC inclusions at 15, 30 and 45 g.kg^{-1} with four inclusion levels of VMP at 0, 1.25, 2.5 and 3.75 g.kg^{-1}.

The model is as shown below:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i\beta_j + e_{ijk}$

Where:

Y_{ijk} = Observation k in i^{th} level of factor A and J^{th} level of factor B

μ = Overall mean population

α_i = Effect of i^{th} level of factor A

β_j = Effect of i^{th} level of factor B

$\alpha_i\beta_j$ = Effect of interaction of factor A and factor B

e_{ijk} = Random error with mean 0 and variance σ^2

Standard brooding, rearing protocols and immunisation were strictly adhered to, as scheduled for broiler. The weekly feed intake (FI) was recorded by subtracting the left over feed from the feed supplied. Chickens were weighed weekly and accurate records of body weight changes were taken at the beginning and end of the starter (day 1-21) and finisher (day 21-42) phases. The FI and weight gain (WG) records were used to calculate the feed conversion ratio (FCR) (i.e. feed consumption (kg) per kg weight gain) using this formula:

$$\frac{\text{Total feed intake (kg)}}{\text{Weight gain (kg)}}$$

Table 2. Gross composition of basal starter and finisher diets fed to experimental chicken (g.100 g^{-1})

Ingredients	Starter diet (g.100 g^{-1})	Finisher diet (g.100 g^{-1})
Soybean cake	38.00	30.00
Soya oil	2.00	2.00
Wheat offal	7.24	7.24
Table salt	0.25	0.25
Oyster shell	0.50	0.50
Di-calcium phosphate	1.50	1.50
*Vitamin-mineral premix^{k}	-	-
Leaf Meal Composite^{m}	-	-
Methionine	0.15	0.15
Lysine	0.05	0.05
Coccidostat (Lasalocid)	0.06	0.06
Total	99.75	99.75
Calculated nutrients:		
Crude protein (%)	22.08	19.38
Metabolisable energy (kJ.kg^{-1})	12,686.14	13,131.02

*1kg of vitamin-mineral premix contains: Vitamin A 10,000,000IU; Vitamin D3 - 2,000,000IU; Vitamin E - 20,000IU; Vitamin K - 2,250mg; Thiamine B1 - 1,750mg; Riboflavin B2 - 5,000mg; Pyridoxine B6 - 2,750mg; Niacin - 27,500mg; Pantothenic acid - 7,500mg; Biotin - 50mg; Choline chloride - 400g; Antioxidant - 125g; Magnesium - 80 g; Zinc - 50 mg; Iron - 20 g; Copper - 5g; Iodine - 1.2 g; Selenium - 200 mg; Cobalt - 200 mg

k = The dietary layout in Table 1 contains the level of VmP in the respective experimental diets

m = See Table 1 for the corresponding levels of LMC in the respective experimental treatment

Data were subjected to analysis of variance (SAS, 2012). Means were separated using orthogonal contrast of the same software at $\alpha_{0.05}$.

RESULTS

The main effect of varying supplemental VmP on growth performance of broiler chicken at the starter phase is shown in Table 3. The FI of broiler chicks was not significantly affected ($p > 0.05$) by dietary inclusion of supplemental VmP at the starter phase. The FI ranged from 921.37 g in broiler chicks with no dietary inclusion of VmP to 967.25 g in those on 0.25 % supplemental VmP. However, WG in broiler chicks fed diets without VmP (421.90 g) was higher ($p < 0.05$) than in other dietary treatments. The WG of 403.75 g and 403.78 g in chicks on 0.125 % and 0.375 % supplemental VmP, respectively, were similar ($p > 0.05$). Lower ($p < 0.05$) WG of 379.58 g was observed in chicks on 0.25 % dietary VmP supplement. The FCR of 2.35, 2.55 and 2.39 in chicks on 0.125, 0.25 % and 0.375 % levels of VmP inclusions, respectively, were similar ($p > 0.05$) to 2.18 in those on diets without supplemental VmP.

The main effect of varying inclusion levels of LMC on performance of broiler chickens at the starter phase is shown in Table 4. The FI was not significantly affected ($p > 0.05$) by the different dietary supplementation of LMC for broiler starter chickens. However, the 305.61 g WG of chicks on diets without dietary LMC supplement was significantly lower ($p < 0.05$) than in other treatments. Broiler chicks on 1.5 % LMC had 418.57 g of WG, which was higher ($p < 0.05$) than in those on other dietary treatments. The FCR followed the same trend with lower but similar ($p > 0.05$) FCR in chicks on supplemental LMC compared with the higher ($p < 0.05$) FCR of 3.16 for those on diets without LMC.

Effects of interaction of dietary VmP and LMC supplements on performance indices of broiler starter chicks are shown in Table 5. There were significant variations ($p < 0.05$) in WG and FCR. The FI was not significantly affected ($p > 0.05$) by the treatments. The WG of broiler chicks on T_1 (417.88 g) T_2 (417.40 g), T_3 (430.42 g), T_4 (417.94 g), T_7 (415.01 g), T_8 (409.86 g) and T_{10} (423.44 g) were similar ($p > 0.05$) but significantly higher ($p < 0.05$) than those on the control diets T_{13} (305.61 g). Broiler chicks on other five diets (T_{12}, T_{11}, T_9, T_6 and T_5) had similar WG ($p > 0.05$) values ranging from 387.82 g

Table 3. Main effect of dietary supplement of vitamin-mineral premix on performance of starter broiler chickens

VMP Inclusion (%)	0.00	0.125	0.25	0.375	SEM	p value
Feed intake (g/bird/21days)	921.37	949.91	967.25	963.78	12.34	0.055
Weight gain (g/bird/21days)	421.90^{a}	403.75^{ab}	379.58^{b}	403.78^{ab}	5.82	0.041
Feed-conversion ratio	2.18^{b}	2.35^{ab}	2.55^{b}	2.39^{ab}	0.02	0.038

SEM: Standard error of the mean; VMP: Vitamin-mineral premix
Means along the same row with different superscripts are significantly different ($p < 0.05$)

Table 4. Main effect of dietary supplement of leaf meal composite on performance of broiler starter chicks

LMC Inclusion (%)	0.00	1.50	3.00	4.50	SEM	p value
Feed intake (g/bird/21days)	965.19	973.15	941.15	937.94	12.34	0.067
Weight gain(g/bird/21days)	305.61^{b}	418.57^{a}	406.50^{a}	405.71^{a}	5.82	0.003
Feed conversion ratio	3.16^{a}	2.32^{b}	2.32^{b}	2.31^{b}	0.02	0.005

Means with different superscripts along the same row are significantly different ($p<0.05$); SEM: Standard Error of Mean
LMC: Leaf meal composite

Table 5. Effects of interaction of dietary vitamin-mineral premix and leaf meal composite supplementation on performance of broiler starter chicks

VMP (g.100 g^{-1})	LMC (g.100 g^{-1})	FI (g)	WG (g)	FCR
	1.5	982.44	417.88^{a}	2.39^{ab}
0.0	3.0	902.86	417.40^{a}	2.18^{b}
	4.5	878.81	430.42^{a}	2.13^{b}
	1.5	930.49	417.94^{a}	2.25^{b}
0.125	3.0	936.41	394.38^{ab}	2.49^{ab}
	4.5	982.83	398.95^{ab}	2.50^{ab}
0.25	0.0	965.19	305.61^{b}	3.28^{a}
	1.5	953.35	415.01^{a}	2.35^{ab}
0.25	3.0	1012.73	409.86^{a}	2.50^{ab}
	4.5	937.75	387.82^{ab}	2.52^{ab}
	1.5	1026.34	423.44^{a}	2.50^{ab}
0.375	3.0	912.61	404.38^{ab}	2.32^{ab}
	4.5	952.39	405.64^{ab}	2.45^{ab}
P value		0.731	0.033	0.001
SEM		12.27	6.23	0.06

Means with different superscript in the column are significantly different (P < 0.05) LMC = leaf meal composite; FI = feed intake; WG = weight gain; FCR = feed conversion ratio; VMP: Vitamin-mineral premix; SEM: Standard error of mean

(T_9) to 405.64 g (T_{12}). The FCR differed significantly ($p < 0.05$) due to dietary treatments. Similar ($p > 0.05$) FCR of 2.13 (T_3), 2.18 (T_2) and 2.25 (T_4) recorded in chicks on various diets were significantly lower ($p < 0.05$) than 3.28 (T_{13}) in chicks on control diets. Chicks had similar FCR, which ranged from 2.32 in chicks on T_{11} to 2.49 in chicks on T_5.

The main effect of varying dietary levels of supplemental VmP on performance of broiler chickens at the finisher phase is shown in Table 6. There were significant differences ($p < 0.05$) in WG, FI and the FCR as a result of the dietary VmP supplement. The WG of chickens fed 0.00 % supplemental VmP was 971.42 g, which was significantly higher ($p < 0.05$) than in other treatments. The 894.41 g and 889.20 g WG in chickens fed 0.25 and 0.375 % dietary VmP, respectively, were similar ($p > 0.05$) but lower ($p < 0.05$) than in other treatments. The WG of 917.36 g in chickens fed 0.125 % VmP was significantly higher ($p < 0.05$) than in those on 0.25 and 0.375 % VmP but lower ($p < 0.05$) than those on 0.00 % VmP. The FI of 2196.88 g in chickens fed 0.125 % dietary VmP was significantly lower ($p < 0.05$) than in other treatments. There were no significant differences ($p > 0.05$) in the FI of chickens given 0.25 % (2256.97 g) and 0.375 % (2268.83 g) supplemental VmP at the finisher phase. The FCR of 2.41 and 2.43 in chickens fed 0.00 and 0.125 % VmP were significantly lower

Table 6. Main effect of dietary supplement of vitamin-mineral premix on performance of finisher broiler chickens (days 22 - 42)

VMP Inclusion (%)	0.00	0.125	0.25	0.375	P value	SEM
Weight gain (g)	971.42^{a}	917.36^{ab}	894.41^{b}	889.20^{b}	0.043	11.40
Feed intake (g)	2319.17^{a}	2196.88^{b}	2256.97^{ab}	2268.83^{ab}	0.048	17.51
FCR	2.41^{b}	2.43^{b}	2.62^{a}	2.59^{ab}	0.044	0.03

Means with different superscripts are significantly different (P<0.05); SEM = Standard Error of Means; FCR = Feed conversion ratio

Table 7. Main effect of leaf meal composite inclusion on performance of broiler finisher chickens

LMC (%)	0	1.5	3.0	4.5	P value	SEM
Weight gain (g)	926.60	888.52	931.97	925.76	0.062	17.52
Feed intake (g)	2540.88^{a}	2231.47^{b}	2257.75^{b}	2221.19^{b}	0.039	11.39
FCR	2.77^{a}	2.57^{ab}	2.47^{b}	2.46^{b}	0.004	0.03

SEM = Standard Error of Means; Means with different superscripts across rows are significantly different (P < 0.05); FCR = Feed Conversion Ratio; LMC = leaf meal composite

(p < 0.05) than 2.62 and 2.59 in chickens fed 0.25 % and 0.375 % VmP, respectively, at the finisher phase.

The main effect of dietary supplement of LMC on performance of broiler finisher is shown in Table 7. There were significant differences (p < 0.05) in FI and FCR due to the dietary LMC inclusion. The WG of chickens fed varying levels of LMC was not significantly different (p > 0.05) at the finisher phase and ranged from 888.52 g in chickens on 1.5 % dietary LMC inclusion to 931.97 g in those on 3.0 % supplemental LMC. The FI of birds on 0.0 % LMC (2540.88 g) was significantly higher (p < 0.05) than in other dietary treatments. Similar (p > 0.05) FI were observed in chicks on 1.5 % (2231.47 g), 3.0 % (2257.75 g) and 4.5 % (2221.19 g) LMC at the finisher phase. The FCR of chicks fed varying level of LMC showed significant differences (p < 0.05). Chickens fed 0.0 % LMC with FCR of 2.77 was higher (p < 0.05) than in other treatments. However, the FCR of chickens on 3.0 % (2.47) and 4.5 % (2.46) LMC supplements were similar and significantly lower (p < 0.05) than those on 1.5 % (2.57) and 0.00 % (2.77) supplemental LMC.

The effect of interaction of varying inclusion levels of VmP and LMC on performance of broiler chickens at the finisher phase is shown in Table 8. The higher (p < 0.05) WG of 1099.57 g in chickens on T_9 with equally higher (p < 0.05) 2200.00 g FI resulted in the lowest FCR as a result of the interaction of dietary VmP and dietary LMC. Chickens fed T_9 (1099.57 g) had significantly higher (P < 0.05) WG than those on T_6 (825.28 g), T_7 (767.78 g), T_8

Table 8. Effects of interaction of premix and leaf meal composite on the performance of broiler finisher chickens

Treatments	VMP (%)	LMC (%)	WG (g)	FI (g)	FCR
T1		1.50	914.41^{abcd}	2310.37^{ab}	2.54^{abc}
T2	0	3.00	1008.80^{ab}	2346.25^{ab}	2.33^{bc}
T3		4.50	991.03^{ab}	2300.87^{ab}	2.34^{bc}
T4		1.50	958.70^{abc}	2272.50^{ab}	2.38^{abc}
T5	0.125	3.00	968.09^{abc}	2199.37^{b}	2.30^{bc}
T6		4.50	825.28^{bcd}	2118.75^{b}	2.58^{ab}
T13		0.00	926.59^{abcd}	2540.87^{a}	2.76^{ab}
T7	0.25	1.50	767.78^{d}	2180.37^{b}	2.93^{a}
T8		3.00	783.68^{cd}	2106.62^{b}	2.76^{ab}
T9		4.50	1099.57^{a}	2200.00^{b}	2.00^{c}
T10		1.50	913.16^{abcd}	2162.62^{b}	2.40^{abc}
T11	0.375	3.00	967.29^{abc}	2378.75^{ab}	2.46^{abc}
T12		4.50	787.15^{cd}	2265.12^{ab}	2.91^{a}
P value			0.002	0.001	0.001
SEM			41.069	63.15	0.111

SEM = Standard Error of Means; Means with different superscripts in the same colums are significantly different (P < 0.05); Tx = treatment; FCR = Feed Conversion Ratio; WG: Weight gain; FI: Feed intake; LMC: Leaf meal composite; VMP: Vitamin-mineral premix

(783.68 g) and T_{12} (787.15 g), but similar ($P > 0.05$) to chicks on other treatments at the finisher phase. The FI of chickens fed diet T_{13} (2540.87 g) was significantly higher ($p < 0.05$) than those on T_5 (2199.37 g), T_6 (2118.75 g), T_7 (2180.37 g), T_8 (2106.62 g), T_9 (2200.00 g) and T_{11} (2378.75 g) at the finisher phase due to combinations of supplemental VmP and LMC. Significantly higher ($P < 0.05$) FCR was observed in chicks on T_7 (2.93), T_8 (2.76) and T_{13} (2.76) compared to those on T_9 (2.00), T_2 (2.33), T_3 (2.34) and T_5 (2.30).

The relationship between dietary supplementation of LMC and the FCR of broiler chicks at the starter phase is shown in Figure 1. The regression curve showed a linear relationship between dietary LMC inclusion and FCR. A significant optimum FCR of 2.3 was obtained at 3.0 % supplemental LMC ($R^2 = 0.98$). The regression is shown in the equation 1 as follows:

$$Y = 0.21x^2 – 1.416x + 4.46 \quad (R^2 = 0.984) \qquad (1)$$

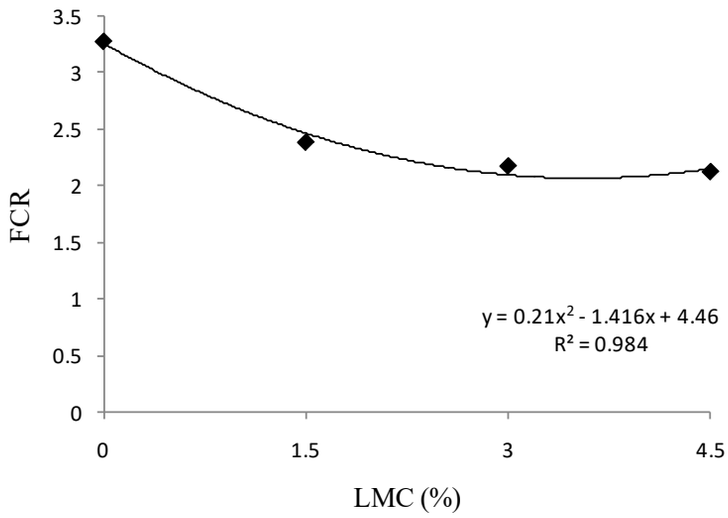

Figure 1. Relationship between leaf meal composite and feed conversion ratio in broiler starter chicks

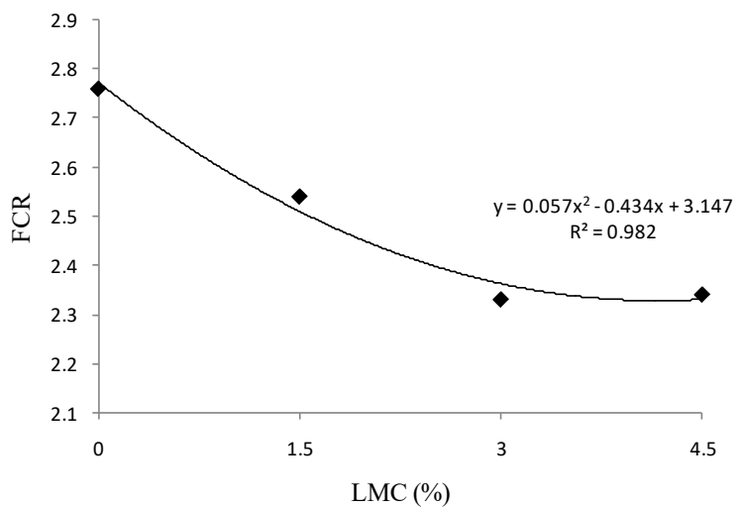

Figure 2. Relationship between leaf meal composite and feed conversion ratio of finisher broiler chickens

The relationship between dietary supplement of LMC and broiler chicken FCR at the finisher phase is shown in Figure 2. The regression curve showed that optimum FCR of 2.38 was obtained with 3 % supplemental LMC level. The regression equation is as shown in the equation 2 below:

$$Y = 0.057x^2 - 0.434x + 3.147 \quad (R^2 = 0.982) \qquad (2)$$

DISCUSSION

The reported reduced weight gain of broiler chickens fed diets containing 3 % moringa leaf meal was attributed to high levels of dietary crude fibre and this lowered FI of chickens (Banjo, 2012). Earlier observations (Ige *et al*., 2006; Olugbemi *et al*., 2010) similarly attributed lower weight gain of chickens to the inherent phyto-chemicals in *Moringa oleifera*. However, other authors (Garcia *et al*., 2007; Nworgu *et al*., 2007; Nkukwana *et al*., 2014) observed positive effects of feeding leaf meal from vegetables to broiler chickens, and the improved performance of broiler chickens was ascribed to gut health improvement. In the present study, the LMC was prepared using four different leaves of vegetables but not just from only moringa. Also, the tested LMC samples were properly air-dried. Broiler chicken, given diets supplemented solely with LMC, had improved performance compared to their counterparts on sole dietary supplement of VmP at the starter phase. This observation suggests that the innate phytonutrients, particularly vitamins and minerals in the LMC, were properly preserved by the drying method and that the innate nutrients were available to the experimental chicks for metabolic processes compared to the commercial VmP. This finding also corroborates the observation of other authors and that dietary LMC positively influenced gut health of broiler chickens (Fasuyi *et al*., 2005; Ihekwumere *et al*., 2008). The reported FCR range of 2.31-2.32 by starter chicks on 3.0 and 4-5 % LMC in this study was higher than reported range of 2.59-2.88 by Odunsi *et al*. (1999).

The FI was not reduced with increasing levels of LMC in broiler diets. However, WG of chickens on 0.00 % (305.61 g) supplemental LMC was significantly lower compared to those on other dietary treatments. Chicks on LMC supplemented diets had similar ($p > 0.05$) weight gain as those on the control diet. This observation may be because the chicks gained more nutrients from the supplemental LMC. Also, the fibre level was relatively higher and the diets supplemented with LMC would elicit earlier satiation compared to those on control diets. This is in agreement with the report of Opara (1996) that leaf meals do not only serve as protein source but also provide other vital bioavailable nutrients like vitamins, minerals and carotenoids.

The FCR in broiler chicken is an important determinant of feed suitability for efficient and profit-orientated production by farmers. A lower FCR meant cheaper rate of production, which will be beneficial to both the consumers and producers; thus, the lower the FCR, the better. The effect of interaction between VmP and LMC on starter broiler chicken performance was significant. All chicks fed diets supplemented with LMC recorded lower FCR than chicks on the control diets. Also, chicks on combined supplemental LMC and VmP had the FCR values which were numerically lower than were recorded in control. This finding suggests that dietary LMC supplementation was the main contributor to the observed difference in FCR. The LMC inclusion in the diets has been reported to improve gut health in broiler chicken (Nworgu *et al*., 2007; Windisch *et al*., 2007; Seyed and Homa, 2011; Nkukwana, *et al*., 2014). The improved FCR observed in this study suggests that LMC may have positively enabled feed utilisation which agrees with the report of Onunkwo and George (2015). The observed improvement in FCR of chicks on sole supplemental LMC or in combination with commercial VmP may also have been due to freshness and properly preserved nutrients in the LMC. The length of storage and handling in manufacturing process may have reduced the potency of commercial dietary VmP supplements (Oyewole *et al*., 2013). Findings in this report are indications that LMC at 3.0 – 4.5 % levels of inclusion would successfully replace dietary VmP in broiler starter chicken diets.

Observations on performance of broiler chickens at the finisher phase indicated that highest FCR was obtained in chickens on 0.25 % dietary VmP supplement. The standard recommendation for optimum performance of commercial broiler chickens (OVN, 2016) ought to be met by the 0.25 VmP provision used for the control diet in this study.

However, earlier reports on effects of dietary supplement of commercial VmP in Nigeria on performance of broiler chickens (Ogunwole *et al*., 2012) and laying pullets (Ojelade and Ogunwole, 2018) showed that commercial VmP were of varying compositions and efficacy. The reports gave credence to vitamin use as production tool rather than just being a nutritional additive solely for disease prevention (Whitehead, 2002; Klasing, 2007; Leeson, 2007). The present recommendation of 0.25 % supplemented VMP may, therefore, be only ideal for chicken raised in optimum conditions (Leeson, 2007; Briz and Perez, 2012) compared to those kept in the open-sided housing type, under which the present research was undertaken. The inclusion of LMC into other dietary treatment groups may have added greater potency to the 0.125 and 0.375 % VmP levels, thus giving a better performance in those groups compared to the control diet, which had only 0.25 % VmP without supplemental LMC. This also in agreement with the popular concept of optimum vitamin-mineral nutrition (OVN, 2016).

Reports have shown that the supplemental LMC contained important vitamins, amino acids and minerals (Makkar and Becker, 1996; Kakengi *et al*., 2005; Mensah *et al*., 2008; Ogbe and Affiku, 2011; Mosuro and Ogunwole, 2019), which could adequately replace the conventional VmP in livestock diets, when used either solely or as leaf composites. According to Ilodibia *et al*. (2016), *Celosia argentea* is a power house of nutrients, with enormous composition of crude protein, fat, zinc, phosphorus, iron, beta carotene and vitamin C – an indication that *Celosia argentea* could contribute significantly to the supply of required nutrients.

The results obtained for finisher chickens had similar trend as in the case of starter chickens. This finding may be because 1.5 % LMC inclusion level in T_1 was the lowest and perhaps inferior to the standard 0.25 % VmP dietary inclusion T_{13}, in terms of efficacy of the inherent vitamins and minerals. Effects of interaction of supplemental dietary VmP and LMC on performance of finisher broiler chickens were significant. There were variations across the different test diet combinations contrary to the trend in the findings for chickens at the starter phase. The highest FCR values were in two of the combined treatment groups, the group with a combination of 0.25 % VmP and 1.5 % LMC in T_7 and those on 0.375 % VmP and 4.5 % LMC in T_{12}. The outcome of the 0.25 % VmP + 1.5 % LMC treatment group was unexpected, especially that the treatment group with a lower 0.125 % VmP and 1.5 % LMC (T_4) and the treatment group with the higher combination of 0.375 % VmP and 1.5 % LMC (T_{10}) had lower FCR than the 0.25/1.5 combination (T_7) test group of chickens. No logical reason could be adduced to this outcome, because all the chickens were under the same ambient conditions and the only variation was in the dietary composition.

The higher FCR of chickens on T_{12} may possibly be attributed to hypervitaminosis syndrome, which may have occurred with higher than the recommended 0.25 % level of VmP in combination with highest level of LMC at 4.5 %. This dietary combination had the highest level of vitamins and minerals among all the treatment groups. Hypervitaminosis is a term, which refers to the body reactions and physiological disorderliness incident on the ingestion of excessive vitamins beyond the requirements of the animal, depending on the levels and the type of vitamins. Symptoms qualifying the toxicity in broiler chickens ranged from mild to severe in nature. Hypervitaminosis in broiler chickens is rare and only a few reports are available (Tang *et al*., 1984; Hamdoon and Rahman, 1990; Surai, 2002). Information on hypervitaminosis of different vitamins does not seem to match the FCR values in this study.

The regression of LMC inclusion on FCR in this study shows that an optimum FCR was obtained at 3.00–4.5 % sole dietary LMC inclusion; while the combination of 0.25 % dietary VmP + 4.5 % LMC in T_9 resulted in the lowest FCR of 2.0 across all the treatment groups. The higher LMC inclusion at 3.0 % or above resulted in a lower FCR. Alahyari-Shahrasb (2012) reported lower performance of broiler chicken without effect on immunocompetence with withdrawal of VmP at the end of finisher phase (day 36-42). Supplemental VmP removal from the starter and finisher chicken on corn-soybean meal diets was noted to decrease weight gain in the Arbor Acres broiler strain (Ogunwole *et al*., 2011). Other authors (Skinner *et al*., 1992; Maiorka *et al*., 2002; Khajali *et al*., 2006) reported varied effects of VmP removal from the diets of broilers on FI, growth performance, carcass yield and FCR. Ogunwole *et al*. (2015) concluded that profitable production of broiler chickens could not be undertaken without dietary supplement of VmP. The authors reported

100 % mortality in broiler chicken raised on VmP-free diets. The role of VmP in broiler starter diets can therefore not be overestimated.

The lowest FCR of 2.0 was obtained in chicken on 0.25 % VMP in combination with 4.5 % LMC in T_9. This observation agrees with the statement of Barroeta *et al*. (2012) that the OVN concept must be engaged in efficient livestock performance and productivity. The question mark on the adequacy of 0.25 % supplemental VmP for broiler chickens becomes valid with this result, as the treatment groups with graded levels of LMC in combination with VmP at 0.125 and 0.25 % showed improved performance. Tian *et al*. (2002) assert that the NRC standard recommendations were for the broilers reared with minimum stressors.

The environmental stressors, such as extreme environmental humidity and temperatures at different times of the day, may have not been taken into account. Under the conditions of the stressors experienced by chickens in this study, the supplemental VmP with LMC would have contributed extra vitamin required above the 0.25 % standard VmP threshold, thereby resulting to improved FCR.

Chicken on sole supplement of 4.5 % LMC (T_3) also performed better than the control group. This finding is in agreement with the report of Oyewole *et al*. (2013), which attributed this improvement to the freshness and natural source of the locally produced condiment used in their study.

CONCLUSION

Air-dried LMC made from mixing of equal proportion of *Vernonia amygdalina*, *Celosia argentea*, *Telfaria occidentalis* and *Moringa oleifera* successfully replaced commercial dietary supplement of VmP in broiler chicken diets.

REFERENCES

Abu, O. A., Olaleru, I. F., Oke, T. D., Adepegba, V. A. & Usman, B. (2015). Performance of broiler chicken fed diets containing cassava peel and leaf meals as replacements for maize and soyabean meal. *International Journal of Science and Technology*, 4(4), 169−173.

Achikanu, C. E., Eze-Stephen, P. E., Ude, C. M. & Ugwuokolie, O. C. (2013). Determination of the vitamin and mineral composition of common leafy vegetables in south eastern Nigeria. *International Journal of Current Microbiology and Applied Sciences*, 2(11), 347−353.

Alahyari-Shahrasb, M., Moravjel, H. & Shivazad, M. (2012). Effect of different levels of vitamin premix during finisher period on broiler performance and immunocompetence in battery cage and floor systems. *Cuban Journal of Agricultural Science*, 46(3), 279−285.

Asaduzzman, M., Jahan, M. S., Mondol M. R., Islam, L. A. & Sarkar, A. K. (2005). Efficacy of different commercial vitamin-mineral premixes on productive performance of caged laying pullets. *International Journal of Poultry Science*, 4(8), 589−595.

Banjo, O. S. (2012). Growth and performance as affected by inclusion of *Moringa oleifera* leaf meal in broiler chicks diet. *Journal of Biology, Agriculture and Healthcare*, 2(9), 35−38.

Barroeta, A. C., Gonzalez, G., Sanz, J. & Briz, R. C. (2012). Optimum vitamin nutrition in poultry breeders. *Optimum Vitamin Nutrition*. 5M Publishing, Sheffield, England. Pp: 41−43.

Bolu, S. A. & Balogun, O. O. (2003). Effects of graded levels of iron-fortified locally produced natural vitamin premix on the performance and carcass characteristics of broilers. *Nigerian Journal of Animal Production*, 30(2), 192−196.

Briz, R. C. & Perez, A. B. (2012). Optimum vitamin nutrition in broilers and turkeys. *Optimum vitamin level in the production of quality animal foods*. 5M Publishing, Sheffield, England. Pp: 139−242.

Fasuyi, O. A., Kola, S. O., Fajemilehin, & Aro, S. O. (2005). Nutritional potentials of Siam weed (*Chromolaena odorata*) leaf meal on laying hens: biochemical and haematological implications. *Pakistan Journal of Nutrition*, 4, 336−341.

Gadzirayi, C. T., Masamha, B., Mupangwa, F. & Washaya, S. (2012). Performance of broiler chickens fed on mature *Moringa oleifera* leaf as a protein supplement to soyabean meal. *International Journal of Poultry Science*, 11(1), 5−10.

Garcia, V., Catala-Gregori, P., Hernandez, F., Megias, M. D. & Madrid, J. (2007). Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology and meat yield of broilers. *The Journal of Applied Poultry Research*, 16(4), 555−562.

Hamdoon, A. A. & Rahman, A. S. (1990). A study on hypervitaminosis-A in broiler chicken. *Veterinary Medical Journal*, 38(1), 115−128.

Ige, A. O., Odunsi, A. A., Akinlade, J. A. & Ojedapo, L. O. (2006). Gliricidia leafmeal in layer's diet: effect on performance, nutrient digestibility and economy production. *Journal of Animal Production and Veterinary Advances*, 5(6), 483−486.

Iheukwumere, F. C., Ndubuisi, E. A. & Onyekwere, M. U. (2008). Performance, nutrient utilisation and organ characteristics of broilers fed cassava leaf meal (*Manihot esculenta* Crantz) *Pakistan Journal of Nutrition*, 7(1), 13−16.

Illodibia, C. V., Ewere, F. U., Akachukwu, E. E., Adimonyemma, R. N., Igboabuchi, N. A. & Okeke, F. (2016). Proximate composition, vitamin and anatomical studies in Gomphrena celosiodes. *Annual Research and Review in Biology*, 10(3), 1−6.

Islam, M. S, Bhuiyan, M. E, Begum, I. A, Miah, M. A. & Myenuddin, M. (2004). Effects of vitamin-mineral premix supplementation on body weight and certain haemato-biochemical values in broiler chickens. *Bangladesh Journal of Veterinary Medicine*, 2(1), 45−48.

Kakengi, A. M. V., Shem, M. N., Sarwatt, S. V. & Fujihara, T. (2005). Can *Moringa oleifera* be used as protein supplement to ruminants? *Asian-Australasian Journal of Animal Science*, 18(1) 42−47.

Khajali, F., Asadi, E. K. & Zamani, M. (2006). Effect of vitamin and trace mineral withdrawal from finisher diets on growth performance and immunocompetence of broiler chickens. *British Poultry Science*, 47, 159−162.

Kim, B. G. & Lindemann, M. D. (2007). A new spreadsheet method for the eperimental animal allotment. *Journal of Animal Science*, 85(2), 218−225.

Klasing, K. C. (2007). Nutrition and the immune system. *British Poultry Science*, 48(5), 525−537.

Krokida, M. K. & Maroulis, Z. B. (2007). Effect of drying method on shrinkage and porosity. *Drying Technology*, 15(10) 2441−2458.

Lakshmi B & Vimla, V. (2000). Nutritive value of dehydrated green leafy vegetable powders. *Journal of Food Science and Technology*, 37(5), 465−471.

Leeson, S. (2007). Vitamin requirements: is there basis for re-evaluating dietary specifications? *World's Poultry Science Journal*, 63(2), 255−266.

Maiorka, A., Laurentiz, A. C., Santin, E., Araujo, L. F. & Macari, M. (2002). Dietary vitamin or mineral mix removal during the finisher period on broiler chicken performance. *Journal of Applied Poultry Research*, 11, 121−126.

Makkar, H. P. S. & Becker, K. 1996. Nutritional value and antinutritional component of whole and extracted *Moringa oleifera* leaves. *Animal Feed Science and Technology*, 63, 211−223.

Malik, A. A, Balogun, O. O. & Dikko, A. H. (2010). An evaluation of the effect of storage of a locally produced natural vitamin premix on the performance of laying hens. *Journal of Agriculture, Forestry and the Social Sciences*, 8(1). Doi: 10.4314/joafss.v8i1.64832

Mensah, J. K, Okoli, R. I., Ohaju-Obodo, J. O. & Eifediyi, K. (2008). Phytochemical, nutritional and Medicinal properties of some leafy vegetables consumed by Edo people of Nigeria. *African Journal of Biotechnology*, 7(14), 2304−2309.

Mosuro, A. O. (2018). *Replacement of dietary vitamin-mineral premix with leaf meal composites in broiler chicken diets*. A thesis in the Department of Animal Science, submitted to the Faculty of Agriculture in partial fulfillment for the award of Ph.D., University of Ibadan, Ibadan, Nigeria. 186 pp.

Mosuro, A. O. & Ogunwole, O. A. (2019). Vitamin and provitamin profiles of selected vegetables as affected by storage and different drying methods. *Journal of Agricultural Sciences*, 64(2), 175−188.

Naikwade, P. V. (2014). Effect of drying methods on nutritional value of some vegetables and chlorophyll retention of leafy vegetables. *Proceeding of the National Conference on Conservation of National Resources and Biodiversity for Sustainable Development*, India, 1−8.

Nkukwana, T. T., Muchenje, V., Pieterse, E., Masika, P. J., Mabusela, T. P., Hoffman, L. C. & Dzama, K. (2014). Effect of *Moringa oleifera* leaf meal on growth performance, apparent digestibility, digestive organ size and carcass yield in broiler chickens. *Journal of Livestock Science*, 161, 139−146.

Nworgu, F. C., Ekemezie, A. A. Ladele, A. O. & Akinrolabu, B. M. (2007). Performance of broiler chickens served heat-treated fluted pumpkin (*Telfaria occidentalis*) leaves extract supplement. *African Journal of Biotechnology*, 6(6), 818−825.

Odunsi, A. A., Farinu, G. O., Akinola, J. O. & Togun, V. A. (1999). Growth, carcass characteristics and body composition of broiler chicken fed wild sunflower (*Tithonia diversifolia*) forage meal. *Tropical Animal Production Investigation*, 2(1), 205−211.

Ogbe, A. O. & Affiku, J. P. (2011). Proximate study, mineral and anti-nutrient composition of *Moringa oleifera* leaves harvested from Lafia, Nigeria: Potential benefits in poultry nutrition and health. *The Journal of Microbiology, Biotechnology and Food Sciences*, 1(3), 296−308.

Ogunwole, O. A., Kolade, E. O., Olumide, M. O.,

Akinsoyinu., A. O., Mako, A. A., Abiola- Olagunju, O. & Adebiyi, O. A. (2011). Evaluation of five proprietary vitamin-mineral premixes in Ibadan, Nigeria for broiler production. *Poultry Science Association (PSA) at the Annual AAAP/AVMA Meeting in St. Louis*, MO. pp. 83.

Ogunwole, A. O., Kolade, E. O. & Taiwo, B. A. (2012). Performance and Carcass Characteristics of Broilers Fed Five Different Commercial Vitamin-Mineral Premixes in Ibadan, Nigeria. *International Journal of Poultry Science*, 11(2), 120−124.

Ogunwole, O. A., Solaru, O. & Tewe, O. O. (2015). Effects of serial withdrawal of dietary vitamin mineral premixes on performance, carcass characteristics and organs weight of broiler chickens. *Tropical Animal Production Investgation*, 18(1) 79−87.

Ojelade, A. Y. P. & Ogunwole, O. A. (2018). Effects of housing systems and different dietary proprietary vitamin-mineral premies on performance characteristics and haematology of pullets from weeks 10 to 21. *Nigerian Journal of Animal Science*, 20(2), 127−139.

Olugbemi, T. S., Mutayoba, S. K. & Lekule, F. P. (2010). Effect of *Moringa oleifera* inclusion in cassava based diets fed to broiler chickens. *International Journal of Poultry Science*, 9(4), 363−367.

Onayemi, O. & Badifu, G. I. O. (1987). Effect of blanching and drying methods on the nutritional and sensory quality of leafy vegetables. *Plant Foods for Human Nutrition*, 37(4), 291−298.

Onyimonyi, A. E. & Onu, E. (2009). An assessment of pawpaw leaf meal as a protein ingredient for finishing broilers. International *Journal of Poultry Science*, 8(10), 1995−1998.

Onunkwo, D. N & George, O. S. (2015). Effects of *Moringa oleifera* leaf meal on the growth performance and carcass characteristics of broiler birds. *Journal of Agriculture and Veterinary Science*, 8(3), 63−66.

Opara, C. C. (1996) *Studies on the use of Aklchornia cordifolla leaf meal as feed ingredient in poultry diets. M.Sc. Thesis*. Federal University of Technology, Owerri, Nigeria.

Oyewole, B. O., Olanrewaju, G. & Dafwang, I. I. (2013). Performance of broilers fed premix prepared from locally sourced materials. *Standard Research Journal of Agricultural Science*, 1(2), 17−20.

OVN (2016). *Optimum vitamin Nutrition; DSM Vitamin Supplementation Guidelines 2016 for animal nutrition*. Check vitamin levels in animal feed, 26 pp. https:// www.dsm.com/content/dam/dsm/anh/en_us/document

Rickman, J. C, Bruhn, C. M & Barrett, D. M. (2007). Nutritional comparison of fresh, frozen and canned fruits and vegetables. Part I. Vitamins C and B and phenolic compounds. *Journal of the Science of Food and Agriculture*, 87(6), 930−944.

Sagar, V. R. & Suresh, Kumar. P. (2010). Recent advances in drying and dehydration of fruits and vegetables: a review. *Journal of Food Science and Technology*, 47(15), 10−18.

Seyed, R. H.& Homa, D. (2011). Herbal plants and their derivatives as growth and health promoters in animal nutrition. *Veterinary Research Communications*, 35(3), 169−180.

Skinner, J. T., Waldroup, A. L. & Waldroup, P. W. (1992). Effects of removal of vitamin and trace mineral supplements from grower and finisher diets on live performance and carcass composition of broilers. *Journal of Applied Poultry Research*, 1(3), 280−286.

SMUI. (2018). *Satellite Map of University of Ibadan*, https://latitude.to/articles-by-country/ng/nigeria/15223/university-of-ibadan

Surai, P. F. (2002). *Natural antioxidants in avian nutrition and reproduction*. Nottingham University Press, Nottingham, England.

Tang, K. N, Rowland, G. N. &. Veltmann, J. R (1984). Vitamin A toxicity: comparative changes in bone of the broiler and leghorn chicks. *Avians Diseases*, 29(2), 416−428.

Tesfaye, E., Animut, G., Urge, M. & Dessie, T. (2013). *Moringa olifera* leaf meal as an alternative protein feed ingredient in broiler ration. *International Journal of Poultry Science*, 12(5), 289−297.

Tian, J. Z., Lee, J. H., Kim, J. D., Han, Y. K., Park, K. M. & Han, I. K. (2002). Effects of Different Levels of Vitamin-Mineral Premixes on growth, performance, nutrient digestibility, carcass characteristics and meat quality of growing-finishing Pigs. *Asian-Australasian Journal of Animal Sciences*, 14(4), 515−524.

Uraku, A. J., Oko, A. O., Onuigbo, H. N., Edwin, N., Orji, O. U., Ezeali, C.& Orinya, S. C. (2015). Determination of some vitamins in three selected African Green Leafy Vegetables. *International Journal of Pharmacological and Medical Science*, 5(1), 22−26.

Whitehead, C. C. (2002). Nutrition and poultry welfare. *World's Poultry Science Journal*, 58(3) 349−356.

Windisch, W., Schedle, K., Plitzner, C & Kroismayr, A. (2007). Natural phytobiotics for health of young animals and poultry: mechanisms and application. *Journal of Animal Science*, 86(14), 140−148.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 53, 2020 (3): 122–131

ISSN 1337-9984
E-ISSN 1338-0095

EVALUATION OF THE RABIT CARCASS AND MEAT QUALITY

Juraj ČUBOŇ[1]*, Peter HAŠČÍK[1], Adriana PAVELKOVÁ[1], Marek BOBKO[1], Lukáš HLEBA[2]

[1]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Technology and Quality of Animal Products, Nitra, Slovak Republic
[2]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Microbiology, Nitra, Slovak Republic

ABSTRACT

The aim of the work was to analyze the carcass structure and the quality of the meat and fat oxidation of French Lop and Californian rabbit breeds. Carcass yield of the Californian rabbit breed was 50 % and the French Lop rabbit was 49.9 %. In the thing muscle of the French Lop rabbit, the protein content was 23.85 g.100 g^{-1} and in the *musculus longissimus dorsi* (MLD) − 23.31 g.100 g^{-1}. The Californian rabbit breed had a protein content of 23.30 g.100 g^{-1} in the thigh muscle and 23.67 g.100 g^{-1} in the MLD. The intramuscular fat content of the French Lop rabbit thigh muscle was 0.92 g.100 g^{-1} and the back muscle was 0.99 g.100 g^{-1}. The thigh muscle of the Californian rabbit had an intramuscular fat content of 1.10 g.100 g^{-1} and a back muscle of 1.07 g.100 g^{-1}. The higher content of oleic acid was found in both breeds: in the MLD of French Lop rabbit breed had 38.32 g.100 g^{-1} FAME (fatty acid methyl ester) and in the MLD of Californian rabbit − 39.76 g.100 g^{-1} FAME. In the thigh muscle, the oleic acid content in a French Lop rabbit was of 30.76 g.100 g^{-1} FAME and in a Californian rabbit breed − 39.02 g.100 g^{-1} FAME. The docosahexaeonic acid content in the thigh muscle of the French Lop rabbit was the same as that of the Californian rabbit (0.03 g.100 g^{-1} FAME). Malondialdehyde content, an indicator of fat oxidation, determined after 5 days of maturation in the MLD of both breeds was the same (0.17 mg.kg^{-1}). The content of MDA in the thigh muscle of the French Lop rabbit was 0.17 mg.kg^{-1} and in the Californian rabbit − 0.16 mg.kg^{-1}. To achieve optimal carcass maturity, it is appropriate to fatten the French Lop rabbits to an older age.

Key words: carcass structure; amino acid; fatty acid; malondialdehyde; rabbit

INTRODUCTION

Rabbit meat consumption in the world is the highest in Malta − 7.5 kg per capita, followed by Italy − 5.5 kg and France − 3.0 kg (Para Pa *et al.*, 2015).

The Californian rabbit is very well muscled in front part. The back is muscled, the legs are short and good muscled. Rabbits slaughter weigh is 4.5 to 5 kg (Doušek, 1994). The Californian rabbit is the second most widespread rabbit breed in the world. The basic color is white. Due to its good fertility and excellent maternal properties, it is suitable for hybridization (Zadina *et al.*, 2004). The breed has a gentle skeleton and very good musculature of the hips, back and thighs, this should be reflected in a high carcass yield, which should reach up to 65 %, excellent meat performance. The slaughter weight can reach up to 3.5 − 5 kg (Verhoef − Verhallen, 2013).

The French Lop rabbit is currently bred in a variety of colors, monochromatic or magpie with a mantle, reaching a slaughter weight of 4 kg at the age of 5 months and 5.5 kg at the age of 8 months (Zadina *et al.*, 2004). Rabbit meat is easily digestible and dietary meat. In addition is low in fat and cholesterol contents, it has a low purine and sodium content. It also has an optimal content of zinc, copper, phosphorus, calcium and cobalt (Zadina *et al.*, 2004). The back muscle (*musculus longissimus dorsi*) has a protein content of 22.4 g.100 g^{-1} and

***Correspondence:** E-mail: juraj.cubon@uniag.sk
Juraj Čuboň, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Technology and Quality of Animal Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
Received: February 11, 2020
Accepted: May 15, 2020

the thigh muscle 21.7 g.100 g^{-1}, the fat content in the back muscle is 1.8 g.100 g^{-1} and the thigh muscle 3.4 % g.100 g^{-1} (Dalle Zotte, 2015).

Rabbit meat is richer in lysine (2.12 g.100 g^{-1}), but also leucine (1.73 g.100 g^{-1}), valine (1.19 g.100 g^{-1} compared to other meats), isoleucine (1.15 g.100 g^{-1}), threonine (2.01 g.100 g^{-1}) and phenylalanine (1.04 g.100 g^{-1}) (Hernández and Dalle Zotte, 2010). Rabbit meat has a relatively high contents of polyunsaturated fatty acid, around 60 % of all fatty acids. Due to this fact, rabbit meat faster oxidized during processing and storage time (Dalle Zotte, 2002).

In rabbit meat there are 57 – 59 g.100 g^{-1} FAME of unsaturated fatty acids (Skřivan *et al.*, 2008). About 32.5 g.100 g^{-1} FAME are polyunsaturated fatty acids and monounsaturated 28.5 g.100 g^{-1} FAME. A higher polyunsaturated acid content (34.6 g.100 g^{-1} FAME) is found in the thigh muscle (Hernández and Gondret, 2006). Also, Dalle Zotte (2015) reported 31.9 g.100 g^{-1} FAME of PUFA and 28.3 g.100 g^{-1} FAME of MUFA in the thigh muscle.

There is a high content of palmitic acid, linoleic acid and oleic acid in rabbit fat. The fat of other animal species has a higher oleic and stearic acid content than that of rabbits (Skřivan *et al.*, 2008).

Rabbit meat has a low cholesterol content of about 45 – 90 mg.100 $g^{-1}$of muscle (Skřivan *et al.*, 2008). The average cholesterol content in rabbit meat is 59 mg.100 g^{-1} (Combes, 2004). The most important factor of fat oxidation is the degree of fatty acid saturation. Technological processing of meat, such as grinding, cooking, cutting, can increase the oxidation of polyunsaturated fatty acids to by-products as pentanal, hexanal, 4-hydroxynoneal and malondialdehyde (MDA) (Gray *et al.*, 1996). After long-term storage of frozen meat Corino *et al.* (2007) found malondialdehyde content in the thigh muscle of rabbits at the level of 60 µmol MDA.kg^{-1}.

The aim of the study was to analyze the carcass structure and the quality of the meat and fat oxidation of French Lop and Californian rabbit breeds.

MATERIAL AND METHODS

The experimental groups were represented by the French Lop rabbit (n = 8) and Californian rabbits (n = 8) fattened up to 6 months of age. All rabbits included in the study were fed equally, with the same composition feed and voluminous feed (hay) – *ad libitum*.

At the age of six months and at the end of the fattening period, the rabbits were weighed, slaughtered and deboned. The carcasses were matured for 5 days at 4 °C, and samples were taken from MLD (*musculus longissimus dorsi*) and thigh (middle part of thigh). Basic carcass parameters, basic chemical composition, amino acid content, fatty acids and malondialdehyde (MDA) content were analysed. Samples were taken from the *musculus longissimus dorsi* (MLD) and thigh muscle. Samples were taken from each part of the muscle for each rabbit. Contents of fatty acids and amino acids were analysed in the thigh muscle and *musculus longissimus dorsi*. Malondialdehyde content as an indicator of fat oxidation was analysed after five days of maturation in MLD and thigh muscle.

Analysis of basic meat composition by FTIR method

The samples from MLD and thigh muscle (30 g) were analysed for proximate composition, specifically moisture, protein, intramuscular fat amino acids and fatty acids using spectrometer Nicolet 6700 in g.100 g^{-1}. Fatty acid contents were analysed as fatty acid methyl ester (FAME) in g.100 g^{-1} FAME (Vavrišínová *et al.*, 2019).

Determination of the oxidative stability

The content of malondialdehyde (MDA) was determined by spectrophotometric method. The rate of secondary lipid oxidation is determined as the thiobarbitur number in mg malondialdehyde (MDA) per kg of meat. Sample preparation was performed according to Cubon *et al.* (2019). The obtained data were calculated and the MDA concentration was expressed in mg.kg^{-1} of meat.

Statistical analysis

The data were statistical analysed by the SAS 9.3 software using the application Enterprise Guide 4.2.

RESULTS AND DISCUSSION

The rabbits of the French Lop breed had a weight before slaughter of 4288 g and the Californian rabbits

Table 1. Slaughter parameters of the French Lop and Californian rabbit

Parameters	French Lop				Californian rabbit				T – test
	Mean	SD	SE	CV %	Mean	SD	SE	CV %	
Live weight (g)	4288.000	219.802	109.901	5.12	3427.000	233.103	116.505	6.800	++
Carcass weight (g)	2117.000	196.204	0.09	9.26	1711.000	150.507	75.28	8.79	++
Liver (g)	97.50	8.18	4.09	8.39	62.50	9.39	4.69	15.003	++
Lungs (g)	69.00	100.04	50.02	144.909	17.75	2.62	1.31	14.801	-
Kidney (g)	24.00	1.41	0.70	5.89	18.25	2.36	1.18	12.904	++
Heart (g)	12.75	2.21	1.10	17.39	9.00	0.81	0.40	9.07	+
Carcass yield (%)	49.29	1.21	0.71	2.45	50.00	1.35	0.75	2.70	-

had a significantly (P ≤ 0.01) lower weight of 3427 g (Table 1). Similar to our results, the weight of rabbits of the Californian rabbit breed of 3.4 kg at 6 months of age was also reported by Zadina *et al*. (2004). With the French Lop rabbits they reported a higher weight compared to our results (4.5 kg). The weight of the carcass was 2117 g, while for the French Lop and for the Californian rabbit (1711 g) it was significantly lower.

Carcass yield was significantly lower (49.29 %; P ≤ 0.05) for the French Lop breed than for the Californian rabbit (50.00 %). Carcass yield than in was reported by Verhoef-Verhallen (2013; 65 %). Tůmová *et al*. (2018) reported a higher slaughter yield (58 %) for large breeds at 90 days of age, while for medium breeds a slaughter yield at 90 days of age was 58-59 %.

The weight of the liver was significantly higher (97.50 g; P ≤ 0.01) in the French Lop breed than in the the Californian rabbit breed (62.50 g). Mota-Rojaz *et al.* (2006) reported lower liver weight for large breeds, such as Chinchilla (83.58 g) and medium-sized breed, such as the Californian rabbit (86.9 g). Similarly, Bianospino *et al.* (2006) reported 68.3 g of liver weight in rabbits and Petkova *et al.* (2011) recorded approximately the same average weight of liver (83.72 g).

The average thigh weight was higher in the French Lop rabbit (730.15 g) than the Californian rabbit

Table 2. Carcass structure of the French Lop and Californian rabbit

Parameters	French Lop				Californian rabbit				T – test
	Mean	SD	SE	CV %	Mean	SD	SE	CV %	
Back + Chest (g)	1179.82	142.48	47.47	12.07	910.22	165.28	55.12	18.16	+
Skin (g)	541.25	30.92	15.46	5.71	493.75	61.28	30.64	12.41	-
Head (g)	371.25	30.92	15.46	8.32	275.00	38.07	19.03	13.74	++
Shoulder (g)	207.77	3.77	1.21	1.79	187.22	17.88	5.86	9.55	-
Thigh (g)	730.15	37.85	15.61	5.18	613.77	15.63	5.21	2.54	++
Meat (g)	1878.46	132.51	40.17	7.05	1481.45	167.55	54.20	11.31	++
Meat (%)	88.73	0.99	0.31	1.11	86.58	0.23	0.07	0.27	+
Bones (g)	239.09	39.83	13.11	0.17	229.79	22.93	7.42	0.99	-
Bones (%)	11.27	0.47	0.16	4.17	13.42	0.24	0.08	1.79	++

Table 3. Basic chemical composition of the MLD (g.100 g^{-1}) from the French Lop and Californian rabbit

Parameters	French Lop				Californian rabbit				T – test
	Mean	SD	SE	CV %	Mean	SD	SE	CV %	
Humidity	74.78	0.90	0.45	1.29	74.15	0.62	0.31	0.88	-
Proteins	23.31	0.76	0.38	3.04	23.67	0.46	0.23	1.79	-
Intramuscular fat	0.92	0.25	0.12	27.61	1.10	0.23	0.11	21.59	-
Minerals	0.99	0.05	0.01	5.50	1.07	0.06	0.02	5.67	-

(613.77 g; Table 2). Neirurer and Fik (2018) reported the average weight of the thighs in the Nitra rabbit breed as 542.8 g. The weight of the foreleg was higher in the carcass of French Lop (207.77 g) than in the Californian rabbit (187.22 g).

The net muscle weight without bone was higher in the carcass of French Lop (1878.46 g) than in the Californian rabbit breed (1481.45 g). The percentage of meat from the carcass was 88.73 % in the French Lop and 86.58 % in the Californian rabbit. Zadina *et al.* (2004) reported that the carcass of a headless rabbit contains from 70 to 85 % of pure muscle. The proportion of bone in the carcass of the French Lop was 11.27 % and in the Californian rabbit – 13.42 %.

The moisture content of the MLD from the French Lop breed was 74.78 g.100 g^{-1} and the Californian rabbit breed – 74.15 g.100 g^{-1} (Table 3). No statistically significant differences were found in basic chemical indicators between these breeds. In the MLD of French Lop breed the protein content was 23.61 g.100 g^{-1} and in the Californian rabbit breed it was 23.67 g.100 g^{-1}. Similarly, Dalle Zotte (2015) reported MLD moisture content in the rabbits – 74.6 g.100 g^{-1}, protein content – 22.4 g.100 g^{-1} and fat content – around 1.8 g.100 g^{-1}.

The intramuscular fat content of MLD was higher in the Californian rabbit breed 1.10 g.100 g^{-1} compare with the French Lop breed (0.92 g.100 g^{-1}). Difference in the intramuscular fat content likely to be affected by the breed and the size of the body frame. Martino *et al.* (2016) reported, similarly with our results, a water content of 75.3 g.100 g^{-1}, a protein content of 22.9 g.100 g^{-1} but a lower intramuscular fat content of 0.70 g.100 g^{-1}. The mineral content of MLD in the of French Lop breed was 0.99 g.100 g^{-1} and in the Californian rabbit breed – 1.07 g.100 g^{-1}. Malík (2002) and Combes (2004) reported higher mineral content in the rabbit MLD (1.2 g.100 g^{-1}).

The moisture content of the thigh muscle (Table 4) was higher in the French Lop (74.23 g.100 g^{-1}) than in the Californian rabbit (73.91 g.100 g^{-1}) breed. The protein content of the thigh muscles of the French Lop rabbits was 23.85 g.100 g^{-1} and in the Californian rabbit it was 23.3 g.100 g^{-1}. The intramuscular fat content was statistically significantly ($P \leq 0.01$) higher in the thigh muscle of the Californian rabbit, 1.64 g.100 g^{-1} than in the French Lop rabbit (0.75 g.100 g^{-1}). Dalle Zotte (2015), similarly to our results, reported the moisture content of the thigh of 73.8 g.100 g^{-1}, but found a lower

Table 4. Basic chemical composition of the thigh muscle (g.100 g^{-1}) of the French Lop and Californian rabbit

Parameters	FB				Kal				T – test
	Mean	SD	SE	CV %	Mean	SD	SE	CV %	
Humidity	74.29	1.63	0.81	2.37	73.91	4.32	0.53	18.63	-
Proteins	23.85	1.69	0.84	6.80	23.30	0.13	0.06	0.55	-
Intramuscular fat	0.75	0.15	0.07	21.08	1.64	0.26	0.13	16.19	++
Minerals	1.10	0.04	0.01	3.61	1.15	0.05	0.02	4.34	-

Table 5. Amino acid content of the thigh muscle (g.100 g^{-1}) of the French Lop and Californian rabbit

Parameters	French Lop				Californian rabbit				T – testt
	Mean	SD	SE	CV %	Mean	SD	SE	CV %	
Proteins	23.85	1.69	0.84	6.80	23.30	0.13	0.06	0.55	-
Lysine	1.32	0.33	0.16	25.53	1.43	0.09	0.04	6.67	-
Leucin	1.23	0.29	0.14	24.18	1.33	0.08	0.04	6.67	-
Methionine	0.49	0.10	0.05	21.67	0.57	0.04	0.02	7.44	-
Threonine	0.69	0.14	0.07	20.55	0.73	0.05	0.02	6.86	-
Valine	0.73	0.14	0.07	20.13	0.76	0.03	0.01	4.28	-
Isoleucine	0.60	0.15	0.07	26.30	0.65	0.04	0.02	7.36	-
Histidine	0.63	0.20	0.10	31.99	0.75	0.07	0.03	10.55	-
Phenylalanine	0.64	0.15	0.07	24.50	0.69	0.04	0.02	6.08	-
Cysteine	0.21	0.03	0.01	15.58	0.24	0.02	0.01	8.94	-
Arginine	0.99	0.24	0.12	24.99	1.07	0.07	0.03	6.76	-

protein content (21.7 g.100 g^{-1}) and a higher fat content (3.00 g.100 g^{-1}). This author also stated that the fat content of rabbit meat may be in the range 0.6 – 14.4 %. Tumová (2013) also reported a fat content of 0.6 to 14.5 % in the rabbit meat.

The amino acid content of histidine in the thigh muscle of the Californian rabbit was 0.75 g.100^{-1} and in the French Lop rabbit it was 0.60 g.100^{-1} (Table 5). Among amino acids, the highest content was found in lysine: in the Californian rabbit – 1.43 g.100^{-1} and in the French Lop rabbit – 1.32 g.100^{-1}. The content of cysteine in the thigh muscle of the French Lop rabbit was 0.21 g.100^{-1} and in the thigh muscle of the Californian rabbit – 0.24 g.100^{-1}. In MLD (Table 6), the amino acid content of phenylalanine was 0.65 g.100^{-1} in the Californian rabbit and 0.60 g.100^{-1} in the French Lop rabbit. The lysine content in the MLD of Californian rabbit was 1.34 g.100^{-1} and in the MLD of French Lop rabbit – 1.23 g.100^{-1}.

Hernández and Dalle Zotte (2010) reported higher contents of lysine (2.1 g.100 g^{-1}), lucein (1.7 g.100 g^{-1}), valine 1.1 g.100 g^{-1}and phenylalanine (1.04 g.100 g^{-1}) in rabbit MLD compared to our results. Wognin *et al.* (2018) found lower contents of phenylalanine (0.77 g.100 g^{-1}) and threonine (0.89 g.100 g^{-1}) compared to our results.

Table 7 presents the fatty acid content of the thigh muscle. Oleic acid was the fatty acid with

Table 6. Amino acid content of the MLD (g.100 g^{-1}) of the French Lop and Californian rabbit

Parameters	French Lop				Californian rabbit				T – testt
	Mean	SD	SE	CV %	Mean	SD	SE	CV %	
Proteins	23.31	0.76	0.38	3.04	23.67	0.46	0.23	1.79	-
Lysine	1.23	0.17	0.08	13.84	1.34	0.21	0.10	15.97	-
Leucin	1.15	0.15	0.07	13.33	1.25	0.19	0.09	15.42	-
Methionine	0.49	0.05	0.02	10.21	0.53	0.06	0.03	12.82	-
Threonine	0.66	0.07	0.03	11.87	0.71	0.09	0.04	18.89	-
Valine	0.70	0.05	0.02	8.33	0.71	0.06	0.03	9.31	-
Isoleucine	0.54	0.08	0.04	15.14	0.60	0.10	0.05	17.44	-
Histidine	0.62	0.07	0.03	11.84	0.65	0.10	0.05	16.40	-
Phenylalanine	0.60	0.07	0.03	12.59	0.65	0.09	0.04	14.39	-
Cysteine	0.23	0.02	0.01	11.47	0.25	0.02	0.01	9.41	-
Arginine	0.91	0.13	0.06	14.18	1.01	0.16	0.08	16.02	-

Table 7. Content of intramuscular fat (g.100^{-1}) and fatty acids (g.100 g^{-1} FAME) and MDA (mg.kg^{-1}) in the thigh muscle of the French Lop and Californian rabbit

Parameters	French Lop				Californian rabbit				T – test
	Mean	SD	SE	CV %	Mean	SD	SE	CV %	
Intramuscular fat	0.75	0.15	0.07	21.08	1.64	0.26	0.13	16.19	++
Arachidonic acid	1.85	0.34	0.17	18.58	1.46	0.30	0.15	20.66	-
Conjugated Linoleic acid	0.14	0.01	0.01	4.74	0.12	0.01	0.01	6.47	++
Docosahexaenoic acid	0.03	0.01	0.01	7.75	0.03	0.01	0.01	10.74	-
Docosapentaenoic acid	0.13	0.01	0.01	4.77	0.14	0.01	0.01	6.14	-
Eicosanoic acid	0.58	0.20	0.10	35.20	0.52	0.08	0.04	15.20	-
Eicosapentaenoic acid	0.10	0.01	0.00	18.24	0.10	0.01	0.01	20.75	-
Heptadecanoic acid	0.29	0.06	0.03	21.63	0.31	0.06	0.03	20.75	-
Lauric acid	0.11	0.01	0.01	13.10	0.12	0.01	0.01	4.71	-
Linolenic acid	0.14	0.05	0.02	36.17	0.12	0.02	0.01	17.19	++
Linoleic acid	5.10	1.49	0.74	29.20	5.63	1.09	0.54	19.45	-
Myristic acid	1.42	0.04	0.02	3.31	1.32	0.04	0.02	3.41	+
Oleic acid	30.76	9.86	4.93	32.07	39.02	2.16	1.08	5.54	-
Palmitoleic acid	24.68	0.23	0.13	1.09	24.30	0.36	0.18	1.49	-
Stearic acid	10.49	0.45	0.22	4.37	10.75	0.21	0.10	1.97	-
Vaccenic acid	4.97	0.15	0.075	3.03	4.73	0.09	0.04	2.06	+
Essential fatty acids	9.04	0.58	0.29	6.48	7.36	0.78	0.39	10.71	+
Omega 3 fatty acids	0.42	0.06	0.031	14.93	0.46	0.04	0.02	8.89	-
Omega 6 fatty acids	9.28	2.09	1.047	22.58	9.74	0.54	0.27	5.62	-
MUFA fatty acids	48.88	2.15	1.07	4.40	50.04	1.51	0.75	3.03	-
PUFA fatty acids	12.10	0.69	0.34	5.70	11.51	1.78	0.13	2.37	-
SAFA fatty acids	33.85	1.32	0.66	3.91	35.81	1.78	0.89	4.99	-
MDA	0.17	0.03	0.01	21.05	0.16	0.01	0.01	11.75	-

the highest contents: in the French Lop rabbit – 30.76 g.100 g^{-1} FAME and in the Californian rabbit – 39.02 g.100 g^{-1} FAME were determined. The content of palmitic acid in the thigh muscle was 24.68 g.100 g^{-1} FAME in French Lop rabbit and 24.30 g.100 g^{-1} FAME in the Californian rabbit.

Likewise, Hernandez *et al.* (2008) found the highest content of oleic and palmitic acid in the thigh muscle of rabbits. Banskalieva *et al.* (2000) reported lower oleic acid content (25.4 g.100 g^{-1} FAME) and higher palmitic acid content (27.3.100 g^{-1} FAME) compared to our results. However, the docosahexaenoic acid content was the lowest in both breeds (0.03 g.100 g^{-1} FAME).

A similar content of palmitic acid (26.94 g.100 g^{-1} FAME) and docosahexaenoic acid (0.16 g.100 g^{-1} FAME) in the thigh muscle was also reported by Rasinska *et al*. (2018). However, compared to our results, they found a lower content of oleic acid in the thigh muscle (26.56 g.100 g^{-1} FAME). Ramírez (2005) reported lower oleic acid content in the thigh muscle (23.16 g.100 g^{-1} FAME) compared to our results, but approximately the same content of palmitic acid (25.08 g.100 g^{-1} FAME).

We found a statistically significant ($P \leq 0.01$) difference in linoleic acid content in the thigh muscle of the French Lop rabbit (0.14 g.100 g^{-1}) FAME compared to the Californian rabbit (0.12 g.100 g^{-1} FAME). There was also statistically significant ($P \leq 0.05$) difference in the myristic acid content of 1.42 g.100 g^{-1} FAME for the French Lop rabbit and 1.32 g.100 g^{-1} FAME for the Californian rabbit, and in the vaccenic acid contents for the French Lop rabbit (4.97 g.100 g^{-1}) FAME and Californian rabbit (4.73 g.100 g^{-1}) FAME. Significant ($P \leq 0.01$) differences were observed in the conjugated linoleic acid content in the thigh muscle of French Lop rabbit (0.14 g.100 g^{-1} FAME) and in the Californian rabbit (0.12 g.100 g^{-1} FAME). Differences in the MUFA content

Table 8. Content of intramuscular fat (g.100^{-1}) and fatty acids (g.100 g^{-1} FAME) and MDA (mg.kg^{-1}) in the MLD of the French Lop and Californian rabbit

Parameters	French Lop				Californian rabbit				T – test
	Mean	SD	SE	CV %	Mean	SD	SE	CV %	
Intramuscular fat	0.92	0.25	0.12	27.61	1.10	0.23	0.11	21.59	-
Arachidonic acid	1.96	0.65	0.32	33.08	2.01	0.39	0.19	19.74	-
Conjugated Linoleic acid	0.13	0.02	0.01	14.96	0.14	0.01	0.01	9.43	-
Docosahexaenoic acid	0.03	0.01	0.01	15.78	0.03	0.01	0.01	5.83	-
Docosapentaenoic acid	0.13	0.01	0.01	4.05	0.13	0.01	0.01	8.00	-
Eicosanoic acid	0.55	0.06	0.03	12.50	0.60	0.08	0.04	13.66	-
Eicosapentaenoic acid	0.11	0.01	0.01	8.56	0.11	0.02	0.01	21.71	-
Heptadecanoic acid	0.34	0.04	0.02	13.37	0.33	0.03	0.01	11.52	-
Lauric acid	0.11	0.01	0.01	2.64	0.11	0.01	0.01	4.03	-
Linolenic acid	0.13	0.01	0.01	11.81	0.15	0.01	0.01	5.26	-
Linoleic acid	5.97	0.66	0.33	11.15	5.91	0.86	0.43	14.60	-
Myristic acid	1.34	0.04	0.02	3.10	1.35	0.05	0.02	3.97	-
Oleic acid	38.32	2.78	1.39	7.27	39.76	1.56	0.78	3.93	-
Palmitoleic acid	24.32	0.31	0.15	1.30	24.35	0.20	0.10	0.84	-
Stearic acid	10.70	0.36	0.18	3.38	10.88	0.21	0.10	1.93	-
Vaccenic acid	4.82	0.12	0.06	2.61	4.77	0.15	0.07	3.25	-
Essential fatty acids	8.37	0.97	0.48	11.63	9.39	1.96	0.98	23.45	-
Omega 3 fatty acids	0.46	0.09	0.04	19.55	0.50	0.07	0.03	15.49	-
Omega 6 fatty acids	10.25	0.79	0.39	7.78	10.16	0.12	0.06	1.20	-
MUFA fatty acids	48.90	1.53	0.76	3.13	49.85	1.26	0.63	2.53	-
PUFA fatty acids	11.55	0.41	0.20	3.62	12.33	0.62	0.31	5.04	-
SAFA fatty acids	35.87	1.73	0.86	4.83	35.29	1.12	0.56	3.18	-
MDA	0.17	0.03	0.01	18.68	0.17	0.01	0.005	9.49	-

(48.88 g.100 g^{-1} FAME for French Lop rabbit; 50.04 g.100 g^{-1} FAME for California rabbit), contents of PUFA (12.10 g.100 g^{-1} FAME for French Lop rabbit; 11.51 g.100 g^{-1} FAME for Californian rabbit) and SAFA contents (33.85 g.100 g^{-1} FAME for French Lop rabbit; 35.81 g.100 g^{-1} FAME for Californian rabbit) were not statistically significant.

We also did not find differences in MDA content in the thigh muscle after 5 days of maturation, the MDA content of the French Lop rabbit was 0.17 mg.kg^{-1} and the Californian rabbit 0.16 mg.kg^{-1}.

Chrastinová *et al.* reported levels similar to our results: SAFA – 35.01 g.100 g^{-1} FAME, MUFA – 49.76 g.100 g^{-1} FAME and PUFA – 11.68 g.100 g^{-1} FAME. Otherwise, Xao (2016) reported the following values measured in the thigh muscle of rabbits: at the age of 35 days – content of MUFA – 14.64 g.100 g^{-1} FAME, PUFA – 52.22 g.100 g^{-1} FAME and SAFA – 33.14 g.100 g^{-1} FAME; at the age of 90 days – contents of MUFA – 18.37 g.100 g^{-1} FAME, PUFA – 37.50 g.100 g^{-1} FAME and SAFA – 44.13 g.100 g^{-1} FAME. Omega-6 fatty acids content was 9.74 g.100 g^{-1} FAME in the thigh muscle of French Rabbit, and 9.28 g.100 g^{-1} FAME in the French Lop, and Omega-3 fatty acids content was 0.42 g.100 g^{-1} FAME in the thigh muscle of the French Lop and in Californian Rabbit 0.46 g.100 g^{-1} FAME).

Opposite to our results, Rasinska *et al.* (2018) reported 21.98 g.100 g^{-1} FAME of omega-6 fatty acids and 2.47 g.100 g^{-1} FAME of omega-3 fatty acids in the rabbit thigh muscle.

In the MLD (Table 8) we found the highest content of oleic acid, in the French Lop rabbit it was 38.32 g.100 g^{-1} FAME and in the Californian rabbit 39.76 g.100 g^{-1} FAME. The difference in the content of palmitic acid between the rabbit breeds was not statistically significant, in the French Lop rabbit it was 24.32 g.100 g^{-1} FAME and in the Californian rabbit –

24.35 g.100 g^{-1} FAME. The lowest value was found in the content of docosahexaenoic acid: in the thigh muscle of the French Lop rabbit − 0.03 g.100 g^{-1} FAME and the Californian rabbit it was 0.03 g.100 g^{-1} FAME.

Rasinska *et al.* (2018) reported lower level of oleic acid (27.00 g.100 g^{-1} FAME) as well as palmitic acid (25.63 g.100 g^{-1} FAME) and higher level of docosahexaenoic acid (0.10 g.100 g^{-1} FAME) in thigh muscle compared to our results. The MUFA content in the MLD of the French Lop was 48.9 g.100 g^{-1} FAME, while in the Californian rabbit it was higher (49.85 g.100 g^{-1} FAME). The SAFA content in the MLD of the French Lop was 35.87 g.100 g^{-1} FAME and in the Californian rabbit it was 35.29 g.100 g^{-1} FAME. All the differences in the fatty acid content are likely to be affected by the breed and the size of the body frame.

The PUFA content in the MLD of the French Lop rabbit was 11.55 g.100 g^{-1} FAME and in the Californian rabbit it was 12.33 g.100 g^{-1} FAME. Xue (2016) reported lower MUFA content (16.30 g.100 g^{-1} FAME) but higher PUFA (50.54 g.100 g^{-1} FAME) and SAFA (33.16 g.100 g^{-1}) levels in MLD of rabbits at 35 days of age. However, at 90 days the MUFA content was 20.41 g.100 g^{-1} FAME, PUFA – 32 g.100 g^{-1} FAME, while the SAFA content was higher (46 g.100 g^{-1} FAME).

Rasinska *et al*. (2017) reported the fatty acid content in winter season: SAFA − 41.26 g.100 g^{-1} FAME, MUFA − 28.40 g.100 g^{-1} FAME and PUFA − 16.28 g.100 g^{-1} FAME. The n-6 fatty acid content was 12.42 g.100 g^{-1} FAME and the n-3 fatty acids content was higher (3.78 g.100 g^{-1} FAME). Rasinska *et al.* (2018) reported higher n-6 fatty acid level (25.99 g.100 g^{-1} FAME) and n-3 fatty acid level (3.23 g.100 g^{-1} FAME) in MLD compared to our results.

The MDA content in MLD was the same for both breeds (0.17 mg.kg^{-1}). In the thigh muscle of the French Lop, the MDA content was 0.17 mg. kg^{-1} and in the Californian rabbit it was 0.16 mg. kg^{-1}. Nakyinsige *et al.* (2015) reported MDA content after killing − 0.014 mg.kg^{-1}, at the first day 0.0263 mg.kg^{-1}, and at 7th day − 0.152 mg.kg^{-1}. Nakyinsige *at al.* (2014) also reported MDA content at the slaughter day − 0.014 mg.kg^{-1} and at the 7th day of maturing − 0.15 mg.kg^{-1}.

CONCLUSION

The rabbits of the French Lop breed and Californian rabbit breed were fatted to 6 months of age. The slaughter yield of the Californian rabbit and the French Lop was approximately the same. To achieve optimal carcass maturity, it is appropriate to fatten the French Lop rabbits to an older age. The protein content of the thigh muscle and MLD from the French Lop and the Californian rabbit breeds was approximately identical. The intramuscular fat content of the thigh muscle and the MLD of the French Lop breed was lower than that of the Californian rabbit breed. The MUFA and SAFA contents in the thigh muscle were higher in Californian rabbit breed. The MUFA and PUFA contents in the MLD were higher in the Californian Rabbit breed. The MDA content in the MLD was the same for both breeds.

ACKNOWLEDGMENTS

This work was supported by the KEGA project no. 027SPU-4/2019.

REFERENCES

Banskalieva, V. (2000). Fatty acid composition of goat muscles and fat depots: a review. *Small Ruminant Research*, 37(3), 255−268. https://doi.org/10.1016/s0921-4488(00)00128-0

Bianospino, E., Wechsler, F. S., Fernandes, S., Roça, R. D. O., & Moura, A. S. A. (2006). Growth, carcass and meat quality traits of straightbred and crossbred Botucatu rabbits. *World Rabbit Science*, 237−246. https://doi.org/10.4995/wrs.2006.563

Combes, S. (2004). Nutritional value of rabbit meat: a review. *Productions Animales*, 17(5), 373−383.

Corino, C., Fiego, D. L., Macchioni, P., Pastorelli, G., Di Giancamillo, A., Domeneghini, C., & Rossi, R. (2007). Influence of dietary conjugated linoleic acids and vitamin E on meat quality, and adipose tissue in rabbits. *Meat Science*, 76(1), 19−28. https://doi.org/10.1016/j.meatsci.2006.10.007

Cubon, J., Hascik, P., Pavelkova, A., Tkacova, J., Hleba, L., Bucko, O., Jarosova, A. & Cisarova, M. (2019). Protein degradation and fat oxidation changes in salted meat

processing. *Journal of Microbiology, Biotechnology & Food Science*. https://doi.org/10.15414/jmbfs.2019.9.special.376-379

Dalle Zotte, A. (2002). Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Livestock Production Science*, 75(1), 11–32. https://doi.org/10.1016/s0301-6226(01)00308-6

Dalle Zotte, A. (2015). Rabbit farming for meat purposes. *Animal Frontiers*, 4, 62–67.

Doušek, J. (1994). *Chov králíků pro masnou produkci.* Praha: Apros, 174 s. ISBN 80-901100-3-7.

Hernandez, P. & Gondret, F. (2006). *Rabbit meat quality. Recent advances in rabbit science*, pp. 290. ISBN 92-898-6636.

Hernández, P. (2008). Enhancement of nutritional quality and safety in rabbit meat. *9th World Rabbit Congres*, Verona, Italy, pp. 1359–1363.

Hernández, P. & Dalle Zotte, A. (2010). Influence of diet on rabbit meat quality, 163–178. In: Blas De C. & Wiseman, C. (eds): *Nutrition of the rabbit*. UK: CAB International, pp. 334. ISBN-13: 978-1-84593-669-3. https://doi.org/10.1079/9781845936693.0163

Chrastinová, Ľ., Čobanová, K., Chrenková, M., Formelová, Z., Poláčiková, M., Lauková, A., Plachá, I., Ondruška, Ľ., Bino, E., Pogány Simonová, M., Strompfová, V., Kandričáková, A., Rajský, M., Mlyneková, Z., Bučko, O. & Grešáková, Ľ. (2018). Vplyv aplikácie doplnkov Bioplex-ZN a tymolu na stráviteľnosť živín a kvalitu mäsa králikov. Zborník odborných a vedeckých prác: Aktuálne smery v chove králikov. [Influence of Bioplex-ZN and thymol applied as supplements on nutrient digestibility and meat quality of rabbits. Proceedings: Current trends in rabbit breeding.] Nitra: NPPC-VUŽV, s. 44–45. ISBN 978-80-89162-69-7.

Malík, V. (2002). *Hydina a králiky*. 1. vydanie. [Poultry and rabbits. 1st edition.] Bratislava: Príroda, s. 88–102. ISBN 80-07-00963-9.

Martino, M., Mattioli, S., Farkas, P., Szendrő, Zs., Dal Bosco, A., Ruggeri, S., Matics, Zs., Castellini, C. & Gerencsér, Zs. (2016). Carcass traits and meat quality of growing rabbits in pens with and without different multilevel platforms. *World Rabbit Science*, 24(2), 129–138. https://doi.org/10.4995/wrs.2016.3922

Mota-Rojas D., Reyes A., Becerril-Herrera M., Flores-Pintado S., Alonso-Spilsbury M., Cardona L. A., Lemus-Flores C. (2006). Slaughtering process, carcass yield and cutting process in California and Chinchilla rabbit breeds. *Journal of Food Technology*, 4(1), 86–89.

Nakyinsige, K., Fatimah, A. B., Aghwan, Z. A., Zulkifli, I., Goh, Y. M., & Sazili, A. Q. (2014). Bleeding efficiency and meat oxidative stability and microbiological quality of New Zealand White rabbits subjected to halal slaughter without stunning and gas stun-killing. *Asian-Australasian Journal of Animal Sciences*, 27(3), 406–413. https://doi.org/10.5713/ajas.2013.13437

Nakyinsige, K., Sazili, A. Q., Aghwan, Z. A., Zulkifli, I., Goh, Y. M., Bakar, F. A., & Sarah, S. A. (2015). Development of microbial spoilage and lipid and protein oxidation in rabbit meat. *Meat Science*, 108, 125–131. https://doi.org/10.1016/j.meatsci.2015.05.029

Neirurerová, P., & Fik, M. (2018). Analýza vybraných úžitkových vlastností nitrianskeho králika. Zborník odborných a vedeckých prác: Aktuálne smery v chove králikov. [Analysis of select production characteristics of the Nitra rabbit. Proceedings: Current trends in rabbit breeding.] Nitra: NPPC-VUŽV, 32–33. ISBN 978-80-89162-69-7.

Para Pa, G. S., Wakchaure, R., Sharma, R., Mahajan, T., & Praveen, P. K. (2015). Rabbit meat has the potential of being a possible alternative to other meats as a protein source: A brief review. *International Journal of Pharmacy & Biomedical Research*, 2, 17–19.

Petkova, M., Grigorova, S., & Abadjieva, D. (2011). Biochemical and physiological changes in growing rabbits fed different sources of crude fiber. *Biotechnology in Animal Husbandry*, 27(3), 1367–1378. https://doi.org/10.2298/bah1103367p

Ramírez, J. A., Díaz, I., Pla, M., Gil, M., Blasco, A., & Oliver, M. À. (2005). Fatty acid composition of leg meat and perirenal fat of rabbits selected by growth rate. *Food Chemistry*, 90(1-2), 251–256. https://doi.org/10.1016/j.foodchem.2004.04.001

Rasinska, E., Czarniecka-Skubina, E., & Rutkowska, J. (2018). Fatty acid and lipid contents differentiation in cuts of rabbit meat. *CyTA-Journal of Food*, 16(1), 807–813. https://doi.org/10.1080/19476337.2018.1488000

Rasinska, E., Czarniecka-Skubina, E., Rutkowska, J., Przybylski, W. & Brzozowski, M. (2017). Fatty acid profile of meat of seasonally fed slow-growing rabbits. *Animal Science Papers and Reports*, 35(3), 265–277.

SAS 9.3 (2008). *Enhanced Logging Facilities*, Cary, NC: SAS Institute Inc.

Skřivan, M., Tůmová, E. & Skřivanová, V. (2008). *Chov králíků a kožešinových zvířat*. [Husbandry of rabbits and fur animals.] Praha: Česká zemnědělská univerzita, 247 s. ISBN 978-80-213-0955-5.

Tůmová, E. & Hrstka, Z. (2013). Porovnaní masa nutrií a králíků. *Maso* [Comparison of coypu and rabbit meat. Meat], 24(5), 47–50.

Tůmová, E., Volek, Z. & Chodová, D. (2018). Genové zdroje králíků v ČR a možnosti jejich využití v produkci masa. Zborník odborných a vedeckých prác: Aktuálne smery v chove králikov. [Genetic resources of rabbits in the Czech Republic and the possibilities for their utilization in meat production. Proceedings: Current trends in rabbit breeding.] Nitra: NPPC-VUŽV, s. 9−14. ISBN 978-80-89162-69-7.

Vavrišínová, K., Hozáková, K., Bučko, O., Tkáčová, J., & Bobko, M. (2019). Slaughter characteristics and physical technological parameters of veal from male calves of Holstein and Slovak Simmental breeds. *Journal of Microbiology, Biotechnology & Food Sciences*, 9(3), 634−638.

Verhoef-Verhallen, E. (2013). *Králíci a hlodavci: praktická encyklopedie*. 2. vyd. [Rabbits and rodents: practical encyclopaedia. 2nd edition] Čestlice: Rebo, 296 s. ISBN 978-80-255-0721-6.

Wognin, L. R. M. F., Otchoumou, K. A., Yao, K. F., & Niamké, S. (2018). Improving the nutritive value and sensory quality of rabbit meat by using leafy vegetables as feedstuffs. *Journal of Animal & Plant Sciences*, 36(1), 5812−5824.

Xue, S., He, Z., Tao, X., Lu, J., Xiao, X., Xie, Y., Zheng, L. & LI, H. (2016). Composition of intramuscular phospholipid fatty acids of Inra rabbit at different ages. *Italian Journal of Food Science*, 28(4), 683−696.

Zadina, J., Mach, K., Skřivanová, V., Hejlíček, K. & Majzlík, I. (2004). *Chov králíků*. 1. vyd. [Rabbit breeding. 1st edition.] Praha: Brázda, s.r.o. 208 s. ISBN 80-203-0325-9.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 53, 2020 (3): 132–144

ISSN 1337-9984
E-ISSN 1338-0095

RELATIONSHIP BETWEEN SEASONAL VARIATION IN THE COMPOSITION OF BULK TANK MILK AND PAYMENT BASED ON MILK QUALITY

Marcos BUSANELLO[1]*, Ione Maria Pereira HAYGERT-VELHO[2], Marcos André PIUCO[3], Vanessa Isabel HECK[2], Morgana STÜRMER[2], Luiz Carlos COSMAM[3], João Pedro VELHO[2]

[1]Department of Animal Science, College of Agriculture, "Luiz de Queiroz"/University of São Paulo - ESALQ/USP, Campus Piracicaba, São Paulo, 13418-900, Brazil
[2]Department of Animal Science and Biological Sciences, Federal University of Santa Maria, Campus de Palmeira das Missões, Rio Grande do Sul, 98300-000, Brazil
[3]State Technical School Celeste Gobbato, Palmeira das Missões, Rio Grande do Sul, 98300-000, Brazil

ABSTRACT

Payment programs based on milk quality (PPBMQ) are important in the dairy sector as they enable farmers to improve profitability upon reaching payment based on milk quality (PBMQ). We used data submitted to a PPBMQ from a dairy farm referring to a four-year period (January 2013 – December 2016). Correlation, multiple regression, and principal component analysis were performed. We found significant correlations between PBMQ and fat (r = 0.32), protein (r = 0.51), and total bacterial count (TBC) (r = -0.66), as well as an effect of all studied variables on PBMQ using multiple regression analysis (with somatic cell count [SCC] also affecting PBMQ). Thus, protein and fat positively and SCC and TBC negatively affected PBMQ value. Principal component analysis revealed an inverse relationship between summer and winter months. In summer months, the PBMQ was affected by the increase of TBC and SCC and decrease protein, whereas in winter months, protein increase and TBC and SCC decrease were relevant. A varied behaviour was detected for the remaining months. Milk components (fat, protein, SCC, and TBC) significantly affected the final value the PBMQ paid to the farmer. Moreover, there was seasonal effect on PBMQ, with PBMQ being higher in winter months and lower in summer months. Variation in milk composition and payment due to the seasonality should be considered by farmers to reach higher values of bonuses, and by the dairy sector to plane adequate payment throughout the year.

Key words: dairy science; milk production; multivariate analysis; principal component analysis

INTRODUCTION

In Brazil, farming dairy cattle is one of the main economic activities, reaching a total of 35 billion of litres of milk produced in 2015 (EMBRAPA, 2017). However, the milk produced is of lower quality than that in the other countries (England, Germany, Italy and Canada), mainly with respect to the total bacterial count (TBC) and somatic cell count (SCC) (Cassoli *et al.*, 2016; Cassoli and Machado, 2016). Together with TBC and SCC, milk compounds (fat, protein, and lactose) are very important to dairy companies and industries because all these five variables directly affect the yield of milk products (More, 2009; Geary *et al.*, 2014; Meneghini *et al.*, 2016; Murphy *et al.*, 2016).

The use of payment programs based on milk quality (PPBMQ) constitutes one of the approaches used by dairy companies and industries to sensitise and incentivise the dairy farmers to improve the milk quality. Such programs seek to improve the milk quality via a monetary incentive paid by litre of milk (Busanello *et al.*, 2017a) and are based on the levels of certain milk compounds, mainly SCC, TBC, protein,

***Correspondence:** E-mail: marcosbusanello@hotmail.com
Marcos Busanello, Department of Animal Science, College of Agriculture, "Luiz de Queiroz"/University of São Paulo - ESALQ/USP, Campus Piracicaba, São Paulo, 13418-900, Brazil
Received: March 19, 2020
Accepted: May 14, 2020

and fat. These programs commonly use payment systems with bonuses, penalties, or a mixed system (bonuses and penalties) (Huijps *et al.*, 2010). However, programs with an approach based only on penalties appear to be the most effective in sensitizing the dairy farmers to improve milk quality (Valeeva *et al.*, 2007).

Studies with PBMQ, as the above-mentioned, used only univariate approaches. Although some of them (for example, Busanello *et al.*, 2017a) used multiple regression analysis, that approach many times is associated as a multivariate one, but being a misunderstanding because only one response variable is used (Rencher, 2002). Therefore, in the present study, we used univariate analyses (correlation and multiple regression) in addition to a multivariate analysis (principal component analysis [PCA]). In particular, PCA enables the creation of linear combinations between the original variables while maintaining their multiple inter-relationships, as well as enabling the characterization of observations derived from resultant new variables, which are termed principal components (Manly, 2004). Specifically, we aimed to study the relationship between variables of milk composition (fat and protein) and quality (SCC and TBC) with PBMQ paid to the farmer, as well as to characterize the months of the year in regard to these milk variables and PBMQ through PCA using data from a commercial dairy farm that was submitted to a single PPBMQ.

MATERIAL AND METHODS

We used the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (Sargeant and O'Connor, 2014) statement as a guideline for this research, in which it was designed as an observational retrospective longitudinal study. The data regarding milk composition and quality represent the period of January 2013 until December 2016 and were provided by the Unidade Educativa de Bovinocultura de Leite (UEBL) at the Escola Estadual Técnica Celeste Gobbato (EETCG), which is a technical school of farming/agriculture. This school is located in Palmeira das Missões County (latitude: 27° 53' 58", longitude: 53° 18' 49", and altitude of 639 metres), in the Northwest Region of the Rio Grande do Sul state, Brazil.

The UEBL worked with an integrated crop-livestock system, in an area with 27 hectares that were designated for milk production and herd handling. The herd was composed of an average of 25 lactating dairy cows (of different ages, number of lactations, and days in milk), and all cows were of the Holstein breed. Moreover, an average of 40 additional animals was present in other categories (calves, heifers, and dry cows). The UEBL had the following facilities: milking parlour, feeding shed, and shed for calves and heifers. The milking parlour was a herringbone type with piped milking equipment with four closed-circuit claws and a room for the milk cooler. Feeds offered in the feeding shed were corn silage (*Zea mays*), ryegrass hay (*Lolium multiflorum*), and concentrated feed. Each cow received a quantity of concentrated feed according to its milk production.

The milking was performed twice daily at 5:30 and 16:00 hours, and was performed by the students of the technical school. Pre-dipping, withdrawal of the first three milk jets in the background of a black mug, use of individual paper towels to dry the teats, and post-dipping were implemented. Every 15 days, the California Mastitis Test was performed to detect possible cases of subclinical mastitis.

After milking, the milking equipment was washed out with sanitizer for 5 minutes, and then rinsed with water at 40 °C. In the sequence, a chlorinated detergent alkaline solution (pH > 11) was used to wash out the milking equipment with water at 70 to 75 °C for 10 minutes. Finally, an acid detergent was used (pH < 3) with water at 30 to 35 °C for 5 minutes and a final rinse was made after that.

Cows were maintained in a semi-confinement system where they had access to pasture after milking in the morning (until 11:00 hours) and at 13:30 until 16:00 hours. Moreover, the cows also had access to pastures after the 16:00 hours milking in the summer. In the winter, ryegrass (*Lolium multiflorum*) and double-purpose wheat (BRS Tarumã; *Triticum aestivum*) pastures were used, whereas in the summer, sorghum (*Sorghum bicolor*) and Tifton 85 (*Cynodon spp.*) were used. The pasture area was divided into paddocks and water was available when the cows were in the feeding shed and waiting room.

Cows were handled according to the guidelines of the Program of Good Practices in the Farm as

outlined by the dairy company that purchased the milk from the EETCG. In particular, all the cows were identified with earrings, care was taken in the production and storage of the feeds offered to the animals, and an exclusive area for cows was provided during the pre-partum. Cows were also identified for milking. Cows with a blue collar were in a transition period, those with a yellow collar were producing colostrum, and cows with a red collar were medicated (for example, with antibiotics), indicating that their milk should be discarded.

For milk analysis, two to four bulk tank milk samples were collected by the dairy company that purchased the milk from the EETCG (one sample per week or each 15 days). Milk samples were sent to Serviço de Análise de Rebanhos Leiteiros (SARLE) at the University of Passo Fundo, which is certified by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) of Brazil, where total dry extract (TDE), defatted dry extract (DDE), lactose, protein, and fat were analysed using near-infrared spectroscopy (Bentley 2000, Bentley Instruments, Chaska, MN, USA) according to the ISO 9622. The SCC and TBC were also analysed but using flow cytometry (Somacount 300, Bentley Instruments) according to the ISO 13366-2. The methods cited above are described by INMETRO IEC 17025:2002 considering that the dairy company remunerates according to milk composition and quality.

The dairy company that purchased the milk from EETCG applied the PPBMQ with regard to milk composition (fat and protein) and milk quality (SCC and TBC). The value paid was calculated considering PPBMQ from a payment table provided by the dairy company, where the values of PBMQ were actualized in June 2017 and such values were used as a reference to calculate the PBMQ (Table 1). The PPBMQ was based on a mixed system (bonuses and penalties) for all the variables (fat, protein, SCC, and TBC). Lactose was not included in the payment system and, because of it, this variable was not used in the statistical analysis of our study.

Table 1. Payment by milk quality table with bonus and penalty values based on milk composition and quality*

Variable	Classes	Payment (in R$)[1]	Payment (in milk-equivalent litres)[1]
Fat Content ($g.kg^{-1}$ in milk)[2]	20.00 – 29.90	-0.029	-0.023
	30.00 – 32.90	0.000	0.000
	33.00 – 36.30	0.013	0.010
	36.40 – 39.70	0.028	0.022
	39.80 – 50.00	0.034	0.027
Protein Content ($g.kg^{-1}$ in milk)[2]	20.00 – 24.90	-0.079	-0.062
	25.00 – 28.90	-0.028	-0.022
	29.00 – 30.90	0.000	0.000
	31.00 – 33.80	0.030	0.024
	33.90 – 36.90	0.083	0.066
	> 37.00	0.100	0.079
Somatic Cell Count (× 1000 $cells.mL^{-1}$)	1.00 – 200.00	0.060	0.047
	201.00 – 400.00	0.040	0.032
	401.00 – 500.00	-0.010	-0.008
	> 501.00	-0.030	-0.024
Total Bacterial Count (× 1000 $cfu.mL^{-1}$)	1.00 – 500.00	0.040	0.034
	51.00 – 100.00	0.030	0.024
	101.00 – 200.00	0.000	0.000
	201.00 – 300.00	-0.020	-0.016
	> 301.00	-0.040	-0.032

*Milk composition – fat and protein content, Milk quality – somatic cell count and total bacterial count, [1]In Payment '-' indicates penalty, [2]Average values, original table with the authors.

The values of PBMQ are presented in Reais (R$; Brazilian currency) and in milk-equivalents, a measure that reflects the financial value equal to one litre of milk, which was also used by Martins *et al.* (2003) and Busanello *et al.* (2017a). Milk-equivalents were provided with the intent that readers could extrapolate our results to other currencies and facilitate the understanding of PBMQ data for other countries. Thus, PBMQ values were calculated in milk-equivalents by dividing them by R$ 1.2669, the average price of a litre of milk in Brazil considering the year of 2017 (Center for Advanced Studies in Applied Economics – CEPEA; College of Agriculture "Luiz de Queiroz"/University of São Paulo – ESALQ/USP). The value of R$ 1.2669 represents US$ 0.3969 and € 0.3475, considering the average value of one Real for the dollar and euro for 2017 of US$ 3.1290 and € 3.6462, respectively.

For the statistical analyses, first, an exploratory data analysis was performed using boxplots to find possible outliers and uncommon values for the variables of fat, protein, SCC, TBC, and PBMQ (in R$ and milk-equivalents). Values of minimum, maximum, median, interquartile range, arithmetic and geometric means, and standard deviations were also calculated for the above-mentioned variables.

Subsequently, correlation analysis between milk composition and quality (fat, protein, SCC, and TBC) and PBMQ (in milk-equivalents), aiming to understand their relationships, was performed. Accordingly, the Spearman correlation coefficient (nonparametric method) was used, considering that SCC and TBC were variables that did not present normal distributions, which is an assumption for Pearson correlation analysis.

Next, a multiple regression analysis to verify which variables were significant with respect to the PBMQ paid to the farmer was performed. In addition, this analysis also enables the acquisition of subsequent estimates of PBMQ based on the milk quality and composition. For this, PBMQ in milk-equivalents was used as a response variable, whereas fat, protein, SCC, and TBC were used as predictor variables in a generalized linear mixed model. In such a model, a heterogeneous first-order autoregressive covariance structure was used to model the unequally spaced repeated measurements of the month within the year (we excluded one month, for reasons described in detail in the following section), which presented lesser values for Bayesian information criterion and Akaike information criterion. The final model was as follows (1):

$$y_{ij} = \beta_0 + TBC\beta_1 + SCC\beta_2 + Prot\beta_3 + Fat\beta_4 + \delta_{ij} + \varepsilon_{ij} \quad (1)$$

where, y_{ij} is the value of the PBMQ by litre of milk in milk-equivalents for the month i at the year j, i = 12, and j = 4; β_0 is the intercept, an average value common to all as observations; $TBC\beta_1$ is the fixed effect of the bulk tank *TBC*; $SCC\beta_2$ is the fixed effect of the bulk tank *SCC*; $Prot\beta_3$ is the fixed effect of the bulk tank protein content in milk; $Fat\beta_4$ is the fixed effect of the bulk tank fat content in milk; δ_{ij} is the random effect of the month within the year; and ε_{ij} is the random error.

For the multiple regression analysis, the assumptions of homogeneity of variances, residual normality, linearity, and multicollinearity were tested. Plots of the standardized residuals versus adjusted predicted values were used to test homogeneity of variances, whereas normal probability plots of standardized residuals and Shapiro-Wilk's test (p value = 0.66) were used to test normality, and plots of standardized residuals against predictor variables (fat, protein, SCC and TBC) were used to test linearity (Koop *et al*., 2009). A variance inflation factor (VIF) was used to test multicollinearity, where VIF = 1 indicates that predictor variables are not correlated, VIF between 1 to 5 indicates moderate correlation, and VIF between 5 to 10 indicates high correlation (Cohen *et al*., 1983). The VIF verifies the degree to which one predictor variable can predict the other predictor variables present in the model. Nevertheless, an outlier for the month of October 2014 (value of 0.114 milk-equivalents) was excluded to meet all the assumptions.

Lastly, a PCA was performed aiming to characterize the observations (12 months of the year) using the arithmetic means for the months of the year in the studied period considering the variables of milk composition and quality, and PBMQ. For this analysis, we used all the data; i.e. we used the data for October 2014 in the average calculation. The PCA enables reduction of dimensions using a linear combination between the original variables to create new variables that are termed principal components, which maintain the information of all the original variables (Manly, 2004). A correlation matrix between original variables was used to perform PCA. The two

first principal components were used to construct the *biplot* graph, which presents the original variables and observations together (Gabriel, 1971).

All the analyses were performed using the SAS software (SAS, 9.1 SAS/2012, 2012). Descriptive and exploratory analyses were performed using the SAS PROC MEANS and SAS PROC SGPLOT, respectively. The correlation analysis was performed using SAS PROC CORR, whereas the multiple regression analysis was conducted using SAS PROC GLIMMIX and the assumptions verification was performed using SAS PROC UNIVARIATE and SAS PROC REG (multicollinearity: VIF). Finally, the PCA was performed using the SAS PROC PRINCOMP. Statistical significance was considered at $P < 0.05$.

RESULTS

From the descriptive statistical analysis for protein, fat, SCC, TBC, and PBMQ (Table 2) it can be observed that TBC and SCC presented values considered distant between arithmetic means (TBC = 146 229 cfu.mL^{-1} and SCC = 304 312 cells.mL^{-1}), geometric means (TBC = 51 647 cfu.mL^{-1} and SCC = 285 000 cells.mL^{-1}), and medians (TBC = 35 500 cfu.mL^{-1} and SCC = 285 500 cells.mL^{-1}), which is an indication that such variables not presented normality. In this case, the use of geometric mean or median is more adequate than the arithmetic mean to describe the values of SCC and TBC because they are not affected by outliers, which are common in these

Table 2. Descriptive statistics for variables of milk composition and quality and payment based on milk quality related to the studied period[1]

Variable	N	Arithmetic Mean	Standard Deviation	Minimum	Median	IR	Maximum	Geometric Mean
TBC (cfu.mL^{-1})	48	146 229	211 667	7000	35 500	212 500	811 000	51 647
SCC (cells.mL^{-1})	48	304 312	114 760	139 000	285 500	154 000	696 000	285 000
Fat (g.kg^{-1})	48	33.38	2.29	25.30	33.55	2.30	37.60	33.30
Protein (g.kg^{-1})	48	31.41	1.38	28.30	31.55	2.15	34.70	31.38
PBMQ (R$)	48	0.078	0.043	0.000	0.076	0.080	0.145	0.077
PBMQ (milk-equivalent)	48	0.061	0.034	0.000	0.060	0.063	0.114	0.061

[1]Study period – January 2013 to December 2016, N – Number of observations, IR – Interquartile range, TBC – Total bacterial count, SCC – Somatic cell count, PBMQ – Payment based on milk quality.

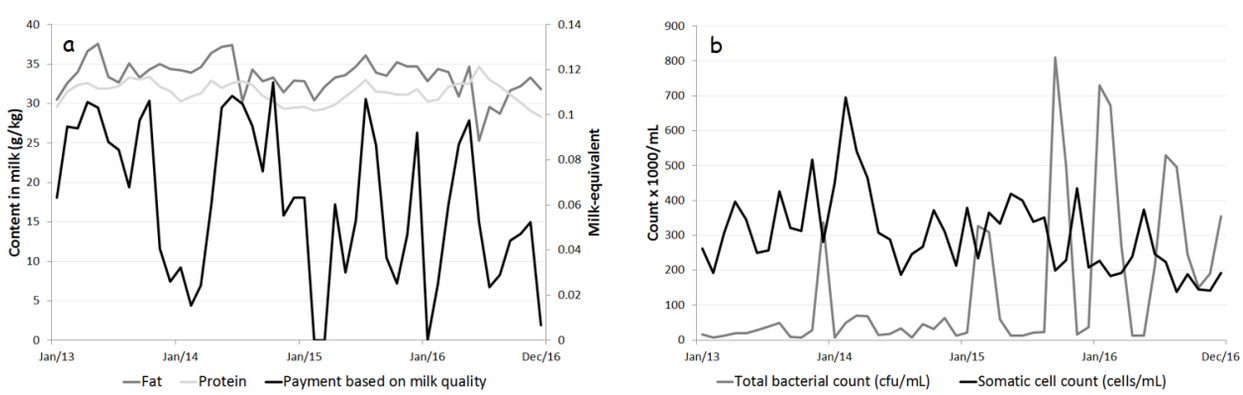

Figure 1. Descriptive behaviour for: (a) the variables of milk composition (fat and protein content) and payment based on milk quality (in milk-equivalents), and for (b) the variables of milk quality (somatic cell count and total bacterial count) over the studied years (January 2013 to December 2016)

variables. Considering the other variables, the average of PBMQ during the studied period was R$ 0.078 and 0.061 milk-equivalents, whereas the mean of protein and fat content in milk was 31.41 and 33.38 g.kg^{-1}, respectively. Interquartile range is the range of the middle 50 % of the data and is a dispersion measure often used with the median and it is indicating that the dispersion seems to be higher for TBC than for the other variables. Moreover, fat and protein content presented a more stable behaviour over the years studied than PBMQ, which exhibited greater variation over the months (Figure 1a). In comparison, SCC had a more stable behaviour than TBC, which presented notable peaks of increase in months of the year 2016, possibly reflecting a high rate of latent mastitis (Figure 1b).

From the multiple regression analysis, considering the PBMQ paid to the farmer and the milk quality and composition, we found that all the variables (fat, protein, SCC, and TBC) significantly affected the PBMQ received (p-value <0.0001 for all) (Table 3). Moreover, the model presented a root mean squared error value of 0.013 milk-equivalents and a determination coefficient (R^2) of 0.85, which is a good measure indicating that most of the variation in the PBMQ (85 %) is due to the milk composition and quality. The multiple regression analysis also provides a model that enables an estimation of the possible PBMQ paid considering the milk composition and quality. Such a model is below (2):

$$PBMQ = -0.368 - (1.110^{-7} \times TBC) - (1.640^{-7} \times SCC) + (0.005 \times Fat) + (0.010 \times Prot) \quad (2)$$

where *PBMQ* is the payment based on milk quality (milk-equivalents); *TBC* is the bulk tank total bacterial count (cfu.mL^{-1}); *SCC* is the bulk tank somatic cell count (cells.mL^{-1}); *Fat* is the % of fat in the bulk tank milk; and *Prot* is the % of protein in the bulk tank milk.

Table 3. Multiple regression analysis estimates (± standard error) related to payment based on milk quality (in milk-equivalent) and milk composition and quality

Variable	Estimated parameter	Standard error	P-value	R^2
Intercept	-0.368	0.052	< 0.0001	0.85
TBC (cfu.mL^{-1})	-1.110^{-7}	1.045^{-8}	< 0.0001	
SCC (cells.mL^{-1})	-1.640^{-7}	2.010^{-8}	< 0.0001	RMSE[3]
Fat (g.kg^{-1})	0.005	0.001	< 0.0001	0.013
Protein (g.kg^{-1})	0.010	0.001	< 0.0001	

TBC – Total bacterial count, SCC – Somatic cell count, R^2 – Determination coefficient, RMSE – Root mean squared error.

Table 4. Spearman's correlation coefficients for variables of milk composition and quality and payment based on milk quality

	PBMQ	TBC	SCC	Fat	Protein
PBMQ	1				
TBC	-0.66*	1			
SCC	0.10	-0.41*	1		
Fat[1]	0.32*	-0.29*	0.51*	1	
Protein[1]	0.51*	-0.28	0.13	0.31*	1

PBMQ – Payment based on milk quality (milk-equivalent), TBC – Total bacterial count (cfu.mL^{-1}), SCC – Somatic cell count (cells.mL^{-1}), [1]Values in g.kg^{-1}, *Significant correlation at the level of 5 % of probability.

In regards to the correlation coefficients (Table 4), TBC, fat and protein showed significant correlation with PBMQ. Protein and fat presented positive correlation (r = 0.51 and r = 0.32, respectively), whereas TBC presented negative correlation (r = -0.66). In addition, the variables also showed significant inter-correlations, where SCC presented positive correlation with fat (r = 0.51) and negative correlation with TBC (r = -0.41), whereas fat presented positive correlation with protein (r = 0.31) and negative correlation with TBC (r = -0.29).

In sequence, PCA was then performed, from which the two first principal components were selected. The first principal component represented 50.2 % of the total data variance and showed an eigenvalue of 2.51, whereas the second principal component represented 33.9 % of total data variance and presented an eigenvalue of 1.69 (Table 5). Together, these two principal components explained 84.0 % of the total data variance. Moreover, the first principal component represents an inverse relationship between PBMQ (0.51) and fat (0.43) versus TBC (-0.57); which were the variables with higher loadings into this principal component. The second principal component represents an inverse relationship between PBMQ (-0.42) and protein (-0.54) with SCC (0.59) and fat (0.40), which were the variables with higher loadings into this component.

The *biplot* graph presents a relationship between the variables (fat, protein, SCC, TBC, and PBMQ) considering the averages for the months of the year in the four studied years (Figure 2). The months of January, February, and March (summer months) presented equal characteristics, which were high TBC and SCC with low-protein content and PBMQ. Conversely, the months of June, July, and August (winter months) presented an inverse pattern compared to the summer months, which was high-protein content and PBMQ with low SCC and TBC. May and April presented high-fat content and SCC with low-protein content and TBC, whereas September presented high-protein content and TBC with low-fat content and SCC (inverse behaviour). October and November were the months that were plotted over the axis of the first principal component, remaining near the general average of the observations for this principal component but presenting an inverse relationship whereby October showed high-protein content and PBMQ with low-fat content and SCC, whereas November showed low-protein content and PBMQ with high-fat content and SCC. December was a month that was plotted over the axis of the second principal component axis, remaining near the general average of the observations for this principal component but showing high TBC with low-fat content and PBMQ. In general, a contrast was observed between summer months and winter months, demonstrating a seasonal effect related to the milk composition and quality and the PBMQ.

Table 5. Eigenvalues and eigenvectors related to principal component analysis of variables using the correlation matrix*

Component	Eigenvectors				
	TBC	SCC	Fat[1]	Protein[1]	PBMQ
Component 1	-0.569	0.277	0.431	0.393	0.509
Component 2	-0.161	0.587	0.400	-0.542	-0.419
	Component 1	Component 2			
Eigenvalues	2.51	1.69			
Proportion	50.19 %	33.85 %			
Cumulative	50.19 %	84.04 %			

*Variables include milk composition and quality and payment based on milk quality, TBC – Total bacterial count (cfu.mL^{-1}), SCC – Somatic cell count (cells.mL^{-1}), [1]Values in g.kg^{-1}; PBMQ – Payment based on milk quality (milk-equivalent).

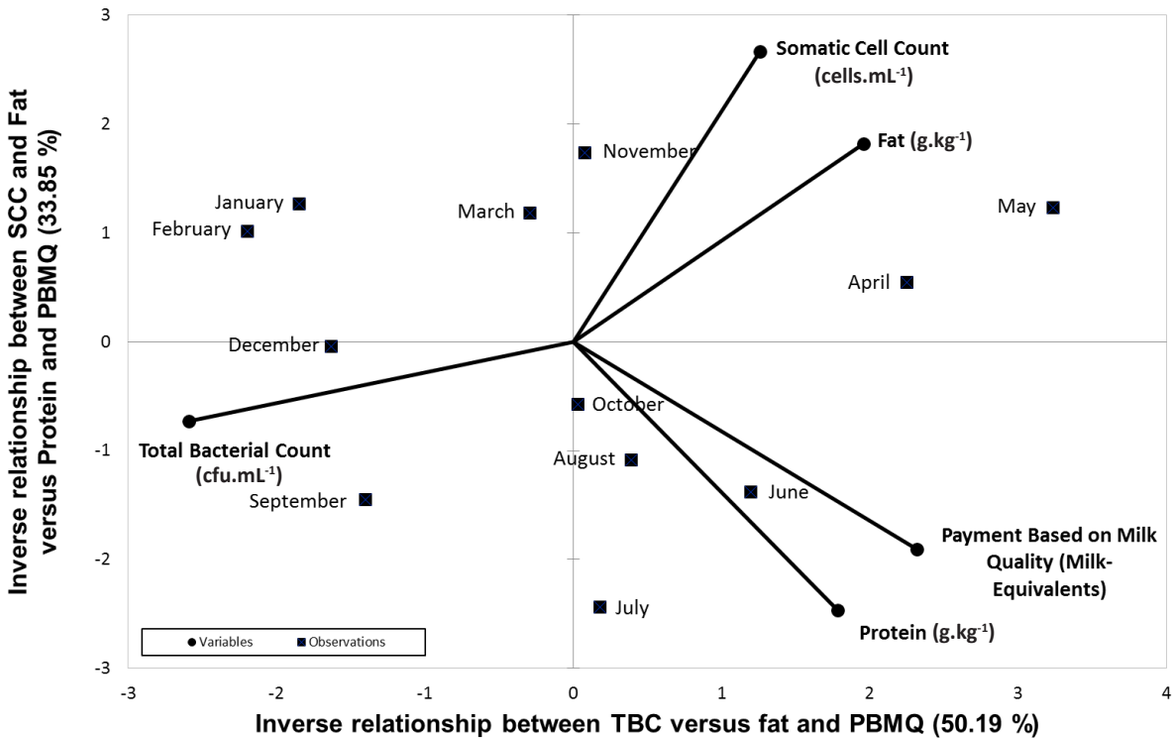

Figure 2. *Biplot* graph obtained from principal component analysis performed using the correlation matrix of the variables of milk composition and quality and the payment based on milk quality. SCC: Somatic cell count (cells.mL^{-1}); PBMQ: Payment by milk quality (milk-equivalents); TBC: Total bacterial count (cfu.mL^{-1})

DISCUSSION

In this study, we aimed to clarify the relationship between variables of milk composition and quality with the PBMQ, taking into consideration the variation caused by seasonality. The data comprised of milk composition, quality and PBMQ from a dairy farm for a four-year period that was submitted to a PPBMQ applied by the dairy company that purchased its milk. The payment table (Table 1) was based in a mixed payment system, where penalties and bonuses were applied together for all the variables included in the program (fat, protein, SCC and TBC). Busanello *et al*. (2017a) analysed information about PPBMQs used by the main dairy companies in Brazil and found that there are no any payment systems in this country based only on penalties. However, some studies have shown that although mixed payment systems (bonuses and penalties together) were effective in motivating and sensitising the farmers to improve milk composition and quality, payment systems based only on penalties were most effective because such systems induced loss aversion (Valeeva *et al*., 2007; Nightingale *et al*., 2008; Huijps *et al*., 2010; Saenger *et al*., 2013).

In Brazil, since 29th December 2011 and after in 26th November 2018, the Normative Instructions 62, 76 and 77 (IN-Brazil) (Brasil, 2011; Brasil, 2018a,b, respectively) established threshold standard values for milk composition and quality. In the Southern Region, maximum geometric mean values for SCC of 500 000 cells.mL^{-1} and TBC of 300 000 cfu.mL^{-1} were recommended as well as minimum arithmetic mean values for a protein content of 29.00 g.kg^{-1} and fat content of 30.00 g.kg^{-1}, considering such measures in a three-month period with one analysis within each month.

In the present study, geometric means for SCC and TBC remained within the thresholds required

by IN-Brazil and, for both, geometric means and medians remained close in comparison with the arithmetic mean, it demonstrated more distant values showing the same behaviour as reported by Busanello *et al.* (2017a) for SCC. In addition, the values of the protein content and fat also remained within the required threshold (31.41 $g.kg^{-1}$ and 33.38 $g.kg^{-1}$, respectively).

Results obtained from multiple regression analysis showed that all the studied variables strongly affected the PBMQ value paid to the farmer. Therefore, our findings demonstrate the possibility to simulate the PBMQ value (milk-equivalents) using equation (2) considering the threshold values recommended by IN-Brazil, it can also demonstrated for some countries such as the United Kingdom (UK) and Northern Ireland (e.g. SCC: 500 000, 180 000, and 195 000 $cells.mL^{-1}$; TBC: 300 000, 30 000, and 17 000 $cfu.mL^{-1}$; protein: 29.00, 32.90, and 32.30 $g.kg^{-1}$; and fat: 30.00, 40.90, and 40.00 $g.kg^{-1}$, respectively for IN-Brazil, UK, and Northern Ireland). Such data were obtained from the National Mastitis Council (NMC, 2013), Cassoli and Machado (2016), Cassoli *et al*. (2016), and the Agriculture and Horticulture Development Board − Dairy (2017) for UK, IN-Brazil (Brasil, 2011; Brasil, 2018a,b) for Brazil, and Department of Agriculture, Environment and Rural Affairs (2016) for Northern Ireland, respectively. From the equation (2) with these values, we obtained PBMQ values of -0.032, 0.144, and 0.133 milk-equivalents for IN-Brazil, UK, and Northern Ireland, respectively.

Such simulation shows us that even the farmers reaching the recommended threshold imposed by the Brazilian government might have a reduction in their milk price because of the PPBMQs. When the quality and composition of Brazilian milk are compared with those of other countries, Brazil still has a considerable limitation to improve milk composition and quality. If Brazilian milk quality were nearer to that of the UK and Northern Ireland, for example, the farmers could attain an increase in their milk price, as was the case in the simulation. However, although milk composition and quality standards exist in Brazil, the farmers that produce and the dairy companies that purchase the milk outside the threshold are not punished in any way by the government. It may be one of the reasons why SCC and TBC improvements have stagnated in the country in recent years (Cassoli and Machado, 2016; Cassoli *et al*., 2016; Busanello *et al*., 2017a,b).

In our study, important correlations between PBMQ and protein, fat, or TBC were found. Increase in the protein and fat content results in an increase in PBMQ, whereas the increase in TBC results in a decrease in PBMQ. Correlations between the other variables are widely found in the literature. For example, the positive relationship between SCC and fat content is a result of the reduction in milk production due to mastitis, concentrating that component in milk (Çinar *et al*., 2015; Stürmer *et al*., 2018). A weak relationship between fat and protein content also was evidenced by Nistor *et al*. (2014).

Multiple linear regression further showed that increase in the SCC results in a decrease in PBMQ as well. Kvapilík *et al*. (2017) also found that SCC, TBC, protein, and fat content affected the milk price paid to the farmers. In contrast, Roma Júnior *et al*. (2009) found a higher PBMQ in autumn, whereas we found higher PBMQ in winter (due to high protein content and low SCC and TBC) and lower PBMQ in summer (due to low protein content and high SCC and TBC). The impact of seasonality on TBC and SCC is heavily discussed in the literature although this primarily involves questions related to hygiene and health of the cows, as well as the influence of the seasons of the year due to the climatic variation (Nightingale *et al*., 2008; Heck *et al*., 2009; More, 2009; Fagan *et al*., 2010; Tančin, 2013; Simioni *et al*., 2014; Hill and Wall, 2015; Tančin *et al*., 2018). Also, there is a relationship between the management of the herds and welfare of the cows, which affect the hygiene of the herds and the milk quality (Sant'Anna and Costa, 2011). Moreover, the practice of withdrawal the first three milk jets can reduce the TBC and SCC in milk (Tančin *et al*., 2006) as it was done in our study.

Fat and protein content are variables that influence positively on PBMQ unlike of SCC and TBC. Thus, fat and protein content favour the bonuses, while SCC and TBC favour the penalties (Roma Júnior *et al*., 2009; Simioni *et al*., 2014). Roma Júnior *et al*. (2009) found that SCC is the main variable causing penalties, while Simioni *et al*. (2014) found that TBC was main variable resulting in penalties. Nevertheless, though PPBMQs induce to an improvement of SCC and TBC, for fat and

protein content other factors are also important (Botaro *et al*., 2013).

The PCA shows an inverse relationship between the summer (January, February and March) and winter months (June, July and August) for protein content, TBC, and PBMQ variables. In the other months, a varied behaviour was observed. The greater advantage of PCA compared with univariate approaches is that PCA considers the multiple relationships between the variables and within the observations (in this case, months), which enables the derivation of more general conclusions.

To our knowledge, no other studies have utilised any multivariate approaches involving a relationship between PBMQ and milk composition and quality. With respect to protein content, Heck *et al*. (2009) also found lower values in the summer (33.90 $g.kg^{-1}$) compared to winter (35.60 $g.kg^{-1}$). In our study, the intake of pastures is favoured in winter because they are based on tempered species, whereas in summer the pastures are based on tropical species. Moreover, the seasons of the year affect the milk production and composition (Fagan *et al*., 2010), with heat stress also having an impact because of the concomitant decrease of nutrient intake (Polsky and von Keyserlingk, 2017). Also, Stürmer *et al*. (2018) found that the climatic variables are responsible for 10.2 % of the variation in composition, quality, pricing, and production of milk.

In general, our results indicate that PBMQ is directly affected by the change in milk composition and quality along the seasons. Consistent with this, Roma Júnior *et al*. (2009) mentioned that seasonality should be considered into the formulation of PPBMQs. Nevertheless, although various countries apply PPBMQs even for other mammalian species as sheep and goats (e.g. France, Italy, Portugal, Greece, and Spain [Pirisi *et al*., 2000; Pirisi *et al*., 2007]), Brazil has only taken small steps to create effective PPBMQs that induce the improvement of bovine milk composition and quality. In addition, few countries include lactose in theirs PPBMQs, such as Ireland and the United States (Sneddon *et al*., 2013), whereas in Brazil lactose has already been identified as showing considerable variation in bulk tank milk samples from dairy farms (variation due to SCC, parity and seasons), which suggests that lactose should also be used in PPBMQs (Alessio *et al*., 2016).

The PPBMQs, together with the production conditions, are determination factors toward improving the profitability and sustainability of the dairy farmers (Michaličková *et al*., 2014; Michaličková *et al*., 2017). Good animal practices that enable the improvement of milk composition and quality lead to improvement of the economic results, mainly due to an increase of the bonuses (Banga *et al*., 2009; Paixão *et al*., 2014; Teixeira Júnior *et al*., 2015). However, effort is required from all the stakeholders within the dairy sector to reach this result, with the lack of reliance between dairy farmers, government, technicians, and dairy companies appearing to constitute one of the most important issues (Devitt *et al*., 2013).

Finally, our study revealed important findings suggesting that PBMQ is influenced by seasonal variation in the milk composition and quality. Nevertheless, our study contains some limitations. The data used represent only the exclusive reality of a single dairy farm for a four-year period. It is possible that the results are not generalisable to those from individual farms and PPBMQs applied by other dairy companies. However, the use of more data from additional farms and dairy companies with different PPBMQs will likely lead to more complex data manipulation and statistical analysis. It is also possible that the effect of different PPBMQs and their results on PBMQ might lead to a different statistical approach. Nevertheless, we consider that these found results are of considerable importance to understand the effects of seasonal variation of milk composition and quality on the PBMQ.

CONCLUSION

Milk compounds (fat, protein, SCC, and TBC) significantly affect the final value of the PBMQ paid to the farmer. Moreover, there is a seasonal effect on PBMQ, wherein winter months (June, July, and August) the PBMQ is higher and in summer months (January, February and March) it is lower. In addition, there is a general negative relationship between SCC and TBC with PBMQ and a positive relationship between protein and fat content with PBMQ. Finally, dairy farmers can increase their PBMQ received in the summer by improving the nutritional management of the herds that enables

an increase in milk protein, as well as improving hygiene and mastitis management to reduce SCC and TBC. Moreover, dairy companies probably should consider a separated formulation of PPBMQs according to the seasons of the year (summer and winter months).

REFERENCES

Agriculture and Horticulture Development Board – Dairy (AHDB – Dairy). (2017). *UK Milk Composition*. https://dairy.ahdb.org.uk/market-information/supply-production/composition-and-hygiene/uk-milk-composition/#.Wi8DnlWnHIV.

Alessio, D. R. M., Thaler Neto, A., Velho, J. P., Pereira, I. B., Miquelluti, D. J., Knob, D. A. & Silva, C. G. (2016). Multivariate analysis of lactose content in milk of Holstein and Jersey cows. *Semina Ciências Agrárias*, 37(4), 2641–2652. http://dx.doi.org/10.5433/1679-0359.2016v37n4SuplI1p2641

Banga, C. B., Neser, F. W. C., Van Der Westhuizen, J. & Garrick, D. J. (2009). Economic values for dairy production traits under different milk payment systems in South Africa. *South African Journal of Animal Science*, 39(5), 112−115.

Botaro, B. G., Gameiro A. H. & Dos Santos, M. V. (2013). Quality based payment program and milk quality in dairy cooperatives of Southern Brazil: an econometric analysis. *Scientia Agricola*, 70(1), 21−26. https://doi.org/10.1590/S0103-90162013000100004

BRASIL. (2011). *Instrução Normativa nº 62, de 29 de dezembro de 2011.* Ministério da Agricultura, Pecuária e do Abastecimento, Secretaria de Defesa Agropecuária.

BRASIL. (2018a). *Instrução Normativa nº 76, de 26 de dezembro de 2018.* Ministério da Agricultura, Pecuária e do Abastecimento, Secretaria de Defesa Agropecuária.

BRASIL. (2018b). *Instrução Normativa nº 77, de 26 de dezembro de 2018.* Ministério da Agricultura, Pecuária e do Abastecimento, Secretaria de Defesa Agropecuária.

Busanello, M., De Freitas, L. N., Winckler, J. P. P., Farias, H. P., Dias, C. T. S., Cassoli, L. D. & Machado, P. F. (2017a). Month-wise variation and prediction of bulk tank somatic cell count in Brazilian dairy herds and its impact on payment based on milk quality. *Irish Veterinary Journal*, 70(1), 1−13. https://doi.org/10.1186/s13620-017-0103-z

Busanello, M., Rossi, R. S., Cassoli, L. D., Pantoja, J. C. F. & Machado, P. F. (2017b). Estimation of prevalence and incidence of subclinical mastitis in a large population of Brazilian dairy herds. *Journal of Dairy Science*, 100(8), 6545–6553. https://doi.org/10.3168/jds.2016-12042

Cassoli, L. D. & Machado, P. F. (2016). Contagem Bacteriana Total (CBT). *Mapa da Qualidade*. Piracicaba. ESALQ/USP 2016. 2nd ed. Clínica do Leite.

Cassoli, L. D., Silva, J. & Machado, P. F. (2016). Contagem de Células Somáticas (CCS). *Mapa da Qualidade*. Piracicaba: ESALQ/USP. 1st ed. Clínica do Leite.

Çinar, M., Serbester, U., Ceyhan, A. & Gorgulu, M. (2015) Effect of somatic cell count on milk yield and composition of first and second lactation dairy cows. *Italian Journal of Animal Science*, 14(1), 3646−3108. https://doi.org/10.4081/ijas.2015.3646

Cohen, J., Cohen, P., West, S. G. & Aiken, L. S. (1983). *Applied multiple regression/correlation analysis for the behavioral sciences*. 3rd ed. New Jersey: Lawrence Erlbaum Associates.

Department of Agriculture, Environment and Rural Affairs. (2016). *Northern Ireland milk quality statistics*. https://www.daera-ni.gov.uk/sites/default/files/publications/dard/2016%20Milk%20Quality%20Stats%20Word%20Document.pdf.

Devitt, C., Mckenzie, K., More, S. J., Heanue, K. & Mccoy, F. (2013). Opportunities and constraints to improving milk quality in Ireland: enabling change through collective action. *Journal of Dairy Science*, 96(4), 2661–2670. https://doi.org/10.3168/jds.2012-6001

EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária). (2017). *Indicadores: Leite e derivados*. 64th ed. EMBRAPA, Juiz de Fora.

Fagan, E. P., Jobim, C. C., Calixto Júnior, M., Da Silva, M. S. & Dos Santos, G. T. (2010). Environmental and handling factors on the chemical composition of milk in dairy farms of Paraná State, Brazil. *Acta Scientiarum Animal Sciences*, 32(3), 309−316. https://doi.org/10.4025/actascianimsci.v32i3.8570

Gabriel, K. R. (1971). The biplot graphic display of matrices with application to principal component analysis. *Biometrika*, 58(3), 453−467. https://doi.org/10.2307/2334381

Geary, U., Lopez-Villalobos, N., O'Brien, B., Garrick, D. J. & Shalloo, L. (2014). Estimating the impact of somatic cell count on the value of milk utilising parameters obtained from the published literature. *Journal of Dairy Research*, 81(2), 223−232. https://doi.org/10.1017/S0022029914000053

Heck, J. M. L., Van Valenberg, H. J. F., Dijkstra, J. & Van Hooijdonk, A. C. M. (2009). Seasonal variation in the

Dutch bovine raw milk composition. *Journal of Dairy Science*, 92(10), 4745−4755. https://doi.org/10.3168/jds.2009-2146

Hill, D. L. & Wall, E. (2015). Dairy cattle in a temperate climate: the effects of weather on milk yield and composition depend on management. *Animal*, 9(1), 138−149. https://doi.org/10.1017/S1751731114002456

Huijps, K., Hogeveen, H., Antonides, G., Valeeva, N. I., Lam, T. J. G. M. & Oude Lansink, A. G. J. M. (2010). Sub-optimal economic behaviour with respect to mastitis management. *European Review of Agricultural Economics*, 37(4), 553−568. https://doi.org/10.1093/erae/jbq036

Koop, G., Nielen, M. & Van Werven, T. (2009). Bulk milk somatic cell counts are related to bulk milk total bacterial counts and several herd-level risk factors in dairy goats. *Journal of Dairy Science*, 92(9), 4355−4364. https://doi.org/10.3168/jds.2009-2106

Kvapilík, J., Hanuš, O., Roubal, P., Říha, J., Urban, P., Jedelská, R., Seydlová, R., Klimešová, M. & Kopunecz, P. (2017). Somatic cells in bulk samples and purchase prices of cow milk. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 65(3), 879−892. https://doi.org/10.11118/actaun201765030879

Manly, B. F. (2004). *Multivariate Statistical Methods: A primer*. 3rd ed. Laramie: Taylor & Francis.

Martins, G. A., Madalena, F. E., Bruschi, J. H., Costa, J. L. & Monteiro, J. B. N. (2003). Economic objectives for dairy cattle selection in a demonstration farm in the Zona da Mata de Minas Gerais. *Revista Brasileira de Zootecnia*, 32(2), 304−314. https://doi.org/10.1590/S1516-35982003000200008

Meneghini, R. C. M., Cassoli, L. D., Martines Filho, J. G., Xavier, C. E. O., Santos, M. V., Caixeta Filho, J. V., Natel, A. S. & Machado, P. F. (2016). How can dairies maximize their profits and properly remunerate their dairy farmers? *Scientia Agricola*, 73(1), 51−61. https://doi.org/10.1590/0103-9016-2014-0261

Michaličková, M., Krupová, Z. & Krupa, E. (2014). Determinants of economic efficiency in dairy cattle and sheep. *Slovak Journal of Agricultural Science*, 47(1), 39−50.

Michaličková, M., Krupová, Z. & Krupa, E. (2017). Economics of dual-purpose dairy cattle under various milk pricing systems. *Bulgarian Journal of Agricultural Science*, 23(2), 183−188.

More, S. J. (2009). Global trends in milk quality: implications for the Irish dairy industry. *Irish Veterinary Journal*, 62(4), 5−14. https://doi.org/10.1186/2046-0481-62-S4-S5

Murphy, S. C., Martin, N. H., Barbano, D. M. & Wiedmann, M. (2016). Influence of raw milk quality on processed dairy products: How do raw milk quality test results relate to product quality and yield? *Journal of Dairy Science*, 99(12), 10128−10149. https://doi.org/10.3168/jds.2016-11172

National Mastitis Council. (2013). *52nd Annual Meeting*. San Diego: NMC.

Nightingale, C., Dhuyvetter, K., Mitchell, R. & Schukken, Y. (2008). Influence of variable milk quality premiums on observed milk quality. *Journal of Dairy Science*, 91(3), 1236–1244. https://doi.org/10.3168/jds.2007-0609

Nistor, E., Bampidis, V. A. & Pentea, M. (2014). Production traits of Romanian Simmental cows at first lactation. *Slovak Journal of Animal Science*, 47(3), 132−141.

Paixão, M. G., Lopes, M. A., Pinto, S. M. & De Abreu, L. R. (2014). Economic impact of the implementation of good agricultural practices related to milk quality. *Revista Ceres*, 61(5), 612−621. https://doi.org/10.1590/0034-737X201461050003

Pirisi, A., Lauret, A. & Dubeuf, J. P. (2007). Basic and incentive payments for goat and sheep milk in relation to quality. *Small Ruminant Research*, 68(1-2), 167−178. https://doi.org/10.1016/j.smallrumres.2006.09.009

Pirisi, A., Sanna, A. & Caria, A. (2000). Raw materials: quality and pricing. *Bulletin of the International Dairy Federation*, no 354.

Polsky, L. & Von Keyserlingk, M. A. G. (2017). Invited review: Effects of heat stress on dairy cattle welfare. *Journal of Dairy Science*, 100(11), 8645−8657. https://doi.org/10.3168/jds.2017-12651

Rencher, A. C. (2002). *Methods of Multivariate Analysis*. 2nd ed. New York: John Wiley & Sons, Inc.

Roma Júnior, L. C., Montoya, J. F. G., Martins, T. T., Cassoli, L. D. & Machado, P. F. (2009). Seasonability of protein and others milk components related with quality payment program. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 61(6), 1411−1418. https://doi.org/10.1590/S0102-09352009000600022

Saenger, C., Qaim, M., Torero, M. & Viceisza, A. (2013). Contract farming and smallholder incentives to produce high quality: experimental evidence from the Vietnamese dairy sector. *Agricultural Economics*, 44(3), 297–308. https://doi.org/10.1111/agec.12012

Sant'Anna, A. C. & Costa, M. J. R. P. (2011). The relationship between dairy cow hygiene and somatic cell count in milk. *Journal of Dairy Science*, 94(8), 3835−3844. https://doi.org/10.3168/jds.2010-3951

Sargeant, J. M. & O'Connor, A.M. (2014). Issues of reporting in observational studies in veterinary medicine. *Preventive Veterinary Medicine*, 113(3), 323–330. https://doi.org/10.1016/j.prevetmed.2013.09.004

SAS Institute. (2012). 2000. Release 8.02. 2000. Cary: SAS Inst. Inc.

Simioni, F. J., Lopes, L. S., Nespolo, C. R., Stefani, L. M., Bordignon, R. & Bittelbrun, M. S. (2014). Season influence on milk physico-chemical and microbiological aspects in Western Santa Catarina. *Semina Ciências Agrárias*, 35(4), 2033–2046. https://doi.org/10.5433/1679-0359.2014v35n4p2033

Sneddon, N. W., Lopez-Villalobos, N., Hickson, R. E. & Shalloo, L. (2013). Review of milk payment systems to identify the component value of lactose. *Proceedings of the New Zealand Society of Animal Production*, 73, 33–36.

Stürmer, M., Busanello, M., Velho, J. P., Heck, V. I. & Haygert-Velho, I. M. P. (2018). Relationship between climatic variables and the variation in bulk tank milk composition using canonical correlation analysis. *International Journal of Biometeorology*, 62(9), 1663–1674. https://doi.org/10.1007/s00484-018-1566-7

Tančin, V., Kirchnerová, K., Foltys, V., Mačuhová, L. & Tančinová, D. (2006). Microbiological contamination and somatic cell count of bovine milk stripped before and after udder preparation for milking. *Slovak Journal of Animal Science*, 39(4), 214–217.

Tančin, V. (2013). Somatic cell counts in milk of dairy cows under practical conditions. *Slovak Journal of Animal Science*, 46(1), 31–34.

Tančin, V., Mikláš, Š. & Mačuhová, L. (2018). Possible physiological and environmental factors affecting milk production and udder health of dairy cows: A review. *Slovak Journal of Animal Science*, 51(1), 32–40.

Teixeira Júnior, F. E. P., Lopes, M. A. & Ruas, J. R. M. (2015). Effect of payment for milk quality on the profitability of dairy farming. *Revista do Instituto de Laticínios Cândido Tostes*, 70(1), 24–34. https://doi.org/10.14295/2238-6416.v70i1.375

Valeeva, N. I., Lam, T. J. G. M. & Hogeveen, H. (2007). Motivation of dairy farmers to improve mastitis management. *Journal of Dairy Science*, 90(9), 4466–4477. https://doi.org/10.3168/jds.2007-0095

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 53, 2020 (3): 145–159

ISSN 1337-9984
E-ISSN 1338-0095

RATIONALE AND LIMITATIONS OF THE DECISION SUPPORT SYSTEMS FOR DAIRY FARMS: A REVIEW

Miroslav ZÁHRADNÍK

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

ABSTRACT

This review aims to summarize the current knowledge about the logical basis of the decision support systems and highlighting future research and development needs for their effective adoptions by dairy farmers. Thus, an emphasis was given on the barriers to their wider uptake in the farming community. The article investigates scientific and professional literature regarding the decision support system framework, according to different factors affecting dairy farm profitability, such as optimal replacement decisions, reproductive performance, economic efficiency, and mortality rates. Accordingly, the description of the various methods being applied was covered. Special attention was drawn on the sustainability agenda, also linking to the idea of benchmarking farm performance and modeling impacts of different management decisions. Benchmarking helps to identify where strengths and weaknesses lie within a farm business. The decision support tools can be used to run various scenarios in the field of structural and technical change on dairy farms. Moreover, they can be tailored for dairy farms that differ in intensity and scale. The multi-actor approach during the development phase of the tools, also enabling dairy farmers to co-design them, may improve the acceptance of co-created solutions at the farm-level. It is also important to drive scientists and extension specialists to provide better understandable outputs by the sets of specific training.

Key words: decision; tool; dairy farm

INTRODUCTION

It has been estimated that the demand for animal-derived protein may double by 2050 (Henchion *et al*., 2017). The importance of animal-source foods in maintaining the health and nutritional status of inhabitants especially in developing countries with limited supply is well described (Neumann *et al*., 2002; Murphy & Lindsay, 2003; Randolph *et al*., 2007; Smith *et al*., 2012). Principal farm-level sustainability concerns in developing regions currently focus on limited food availability due to low agricultural yields, lack of producer education, and inadequacies of transport and sanitary infrastructure (Godfray *et al*., 2010). A common description of sustainability is the ability of a system, a firm or a sector to survive in the long run. The concept of resilience indicates the ability of a system, firm or sector to maintain its structural and functional capacity after a disturbance or shock (Perrings, 1998). Resilience is evidenced by an ability to recover and persist. According to Garmestani *et al*. (2006) the most resilient industries will be those with functions spread across the range of firm size. This will require breeders to maximise their efficiency and mitigate the negative environmental footprint. Farmers are encouraged to redesign and tailor their livestock farming systems to improve their sustainability (Rogers *et al*., 2004, Leeuwis, 2004). Van Calker *et al*. (2005) divided sustainability into four aspects: economic, internal

***Correspondence:** E-mail: miroslav.zahradnik@nppc.sk
Miroslav Záhradník, NPPC – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic

Received: May 5, 2020
Accepted: September 7, 2020

social, external, social, and ecological sustainability. They selected profitability as the only attribute for measuring economic sustainability in Dutch dairy farming. More decision support systems (DSS) are now being offered for the farming community to accomplish this task (Andrew *et al*., 2013; Tamayo *et al*., 2010; Zhong-xiao & Yimit, 2008; Melville, 2010; Korte *et al*., 2012; Aubert *et al*., 2012).

Rationale of the DSS

Decision support tools can be designed as standard decision support tools used by advisors to discuss the issue with farmers (Stonehouse *et al*., 2002; Castelan-Ortega *et al*., 2003; Cabrera *et al*., 2005; Veysset *et al*., 2005; Crosson *et al*., 2006; Nelson *et al*., 2002; Fraisse *et al*., 2015). They may also be conceived as tools for a participatory discussion among stakeholders of in different production contexts (Bernet *et al*., 2001), or as prospective tools to support policy-making (Pacini *et al*., 2004, Rennings and Wiggering, 1997). For example, greenhouse gas emissions can be modelled and compared between organic and conventional systems (Kustermann *et al*., 2008). Such models may also be conceived as decision support tools for farmers, especially when the main viewpoint is productive (Diaz-Solis *et al*., 2003; Pla *et al*., 2003) or economic (Schaik *et al*., 2001; Bush *et al*., 2008). Other research models are aimed at a better understanding of farm operations and their consequences (Hervé *et al*., 2002; Cournut and Dedieu, Ingrand *et al*., 2003, 2004; Rotz *et al*., 2005; Andrieu *et al*., 2007).

The benefits of using a decision support tool are that it can improve individual productivity, improve decision quality and problem solving, as well as facilitate interpersonal communication. It can also improve decision-making skills and increase organizational control, present the likelihood of various outcomes resulting from different options (e.g. Power, 2002; Turban *et al*., 2007, Rossi *et al*., 2014, Dicks *et al*., 2014; Parker, 2004, Alenljung, 2008), with the support of appropriate information technology (Lindblom *et al*., 2014).

Limitation of the uptake

Despite the obvious advantages the uptake has been limited (Alvarez and Nuthall, 2006; Gent *et al*., 2013; Parker *et al*., 1997). Moreover, the levels of acceptance are low, because scientists fail to capture the actual needs of the farming sector (e.g. McCown, 2002; 2005; Parker & Sinclair, 2001; Öhlmér, 2001: Öhlmér *et al*., Melville, 2010) and many of the decisions are made with inadequate or incomplete datasets (Elhag and Walker, 2011).

Another challenge is to build models that will easily be appropriable by farmers and that will allow them to consider in-depth changes. Building them in a participatory way with farmers could be one way of making them more appropriable (Woodward *et al*., 2008; Cerf *et al*., 2008). Many studies marked user-friendliness or user involvement and effective communication during the development as a critical factor (Harris & Weistroffer 2009, Stewart, *et al*., 2013; Valls-Donderis *et al*., 2013; Volk *et al*., 2010; Jakku and Thorburn 2010; Meensel *et al*., 2012; Hall *et al*., 2010; Whittaker *et al*., 2013; McIntosh *et al*., 2011; McIntosh *et al*., 2008; Nguyen *et al*., 2006; Robinson, 2004; Freebairn, 2002; Hartwick & Barki, 2001; Newman *et al*., 2000). Furthermore, the usability for different users in varying situations and contexts is important (Rogers *et al*., 2011).

Benchmarking farm performance

National level competitiveness refers to the ability of a country to produce goods and services that meet the test of foreign or world market competition, while simultaneously maintain and expand domestic real income (Kaspersson *et al*., 2002). A key indicator in measuring the economic sustainability of an activity is profitability. If profits are negative, the revenues cannot cover the costs, which after some time will lead to bankruptcy of the firm and its closure. Positive profits as such reflect that an economic activity adds value, that what is produced is valued more highly by society than the inputs used for its production (de Jong, 2013). Furthermore, the return on investment can be measured by the improvement in environmental quality or the improvement in productivity of the agri-sector (Shepherd and Wheeler, 2010). The sustainability agenda indeed supports idea of benchmarking farm performance. Benchmarking itself is according to Franks (2003) not particularly radical for a farm manager to improve farm performance. The exact definition may vary but we can conclude that it involves borrowing good ideas from others about how to improve (Brown, 1995). This method requires specific measures of selected key performance

indicators (KPIs) which describe the competitive performance level. More recently, sustainability KPIs are gaining interests too (Iribarren, 2011), while innovations in information and communication technology have opened a window of opportunities for on-line benchmarking via computer or via smartphone (Kaloxylos *et al*., 2014). Moreover, software and reports can be developed with which the indicators are reported back to farmers and added to their "dashboard" for monitoring their farm compared to others (Poppe, 2013). Many authors have already discussed key-issues regarding the design and use of sustainability assessment (e.g. Binder *et al*., 2010; Gasparatos & Scolobig, 2012; Gibson, 2006; Ness *et al*., 2007; Pope *et al*., 2004; Weaver & Rotmans, 2006). A first key-issue is the contested meaning of sustainability and sustainable development (Bond *et al*., 2013; Hopwood *et al*., 2005; Pope *et al*., 2004; Waas *et al*., 2011). As a result, for benchmarking sustainability and farm productivity, there is a need for a well-defined normative dimension of sustainability assessments, including the concept of sustainability (Binder *et al*. 2010). As many authors, Bond and Morrison-Saunders (2013) state the meaning of sustainability should be formulated for every assessment, taking into account the context in which it occurs. Literature reviews also shows that different purposes and levels also suggest different end-users (Van Passel & Meul, 2010).

The numerical integration combining the indicator results to present it as a single index or composite indicator (Gómez-Limón & Riesgo, 2009; Van Passel *et al*., 2007) is also implemented in the EkonMOD milk tool linking to management decisions and strategic choises available for dairy farmer management in Slovak conditions (Zahradnik, 2017; Záhradník and Pokrivčák, 2016a, 2016b; Zahradnik *et al*., 2018). Generally, each of the application under the umbrella of the EkonMOD milk platform is used to evaluate the economic consequences of different on-farm strategies. The interactive dairy farm model approach was developed at farm level and based on a static approach. This modeling framework was built to serve the purposes of a wider research strategy. The main objective of this activities concerns an analysis of possible effects of changing conditions on different Slovak dairy cow operations. The model developed can be used to run various scenarios in the field of structural and technical change on dairy farms. Moreover, it can be tailored for dairy farms that differ in intensity and scale. The associated assessments should apply sound statistical methods connecting also to the added value coming from the academia and research result available. Furthermore, input procedure to the model (application, software) has to be simplified and user friendly. The main argument for increased end-user acceptance will be the farm specific adjustments corresponding with user-selected strategic processes on the dairy operation. The procedural instrument to guide the use of sustainability assessment tools within strategic decision making was developed by Coteur *et al*. (2016) with framework allowing a farm-specific and flexible approach leading to harmonised actions towards sustainable farming. The time dimension in this study were defined by five phases based on the framework of De Ridder *et al*. (2007). They propose an integrated assessment, followed by problem analysis and finding the options, analysis and finally, the follow-up. The purpose of 5 step approach tailored by Coteur *et al*. (2016) is gaining insights on the sustainability of multiple farm aspects and stressing the importance of the distinction between assessing the farm and interpreting the results is essential in this framework as the interpretation of results occurs preferably in different ways and depends on the tool choice. The improvement strategies will be implemented in a fourth step and correspond to the third analysis phase of de Ridder *et al*. (2007) followed by the benchmarking and follow-ups (Coteur *et al*., 2016). According to the outcomes of EIP-AGRI focus group benchmarking of farm productivity and sustainability performance outcomes final report, the macro level benchmarking analysis involves a more generic framework where specific farm conditions related productivity and sustainability are shaped by policy, law and regulation, and trade. In addition, macro conditions are influenced by economic and social developments and demographics, technical advances and environmental conditions. Following the report the use of benchmarking data in the aggregate form may benefit the agricultural industry, and indirectly or directly the farmer, in achieving greater productivity and sustainability. In addition, benchmarking may inform policy development,

guide industry regulators and trade organisations, inform industry research, development and innovation, and provide a wealth of information for advisors and educational institutions. The report also suggests that in general, an ideal macro benchmarking system would give clear indicators on where the greatest impact of policies for encouraging competitiveness, productivity and sustainability could be found (EIP-AGRI, 2016).

Implications for dairy farming

In general, dairy farms are deficient in the use of advanced projection frameworks such as simulations (Bewley *et al*., 2010). Nevertheless, an efficient DSS framework is critical for dairy farming management and decision-making through e.g. optimisation of synthetic systems (Cabrera *et al*., 2006, Booty *et al*., 2009). Herd management practices are essential for the productivity of dairy farms (El-osta and Morehart 2000). Growing the herd brings additional difficulties for the farm management (Gargiulo *et al*. 2018). Moreover, inappropriate farm management can negatively impact health and welfare standards of the productive livestock and lower the economic results of the dairy operation (Calsamiglia *et al*., 2018). Keeping the records on an individual cow level is crucial for optimal management decision if necessary (Barragan *et al*. 2016). However, switching from herd level to a cow level in based on a real-time data acquisition (Debauche *et al*., 2018).

Culling decisions

Optimal replacement decisions are cited as one of the most important factors affecting dairy farm profitability (van Arendonk, 1984), and these decisions are directly affected by fluctuations in milk price, salvage values, and replacement costs. Culling decisions are based primarily on milk production and partially on health status. Despite their economic importance, culling decisions are often made in a nonprogrammed fashion and based partly on the intuition of the decision maker (Lehenbauer and Oltjen, 1998). Little or no effort is made to support replacement decisions using economic or financial methods. Traditionally, culling of dairy cows has been viewed from a historical viewpoint. Many dairy farmers and their consultants calculate their annual culling rate and focus on the percentage of cows culled for a variety of reasons (Eicker and Fetrow, 2003). To improve culling decisions, a more prospective approach is preferable and could result in different culling decisions by producers. High yields of dairy cows frequently contribute to the increased health issues. The consequent costs and labour required decrease the overall farm productivity. Lifelong milk productivity of dairy cows can be referred as crucial indicator for management representatives. Moreover, the m dairy cow's milk yield and the stage of lactation have a significant impact on culling (Rajala-Schultz, 1999). Dairy cows culling and heifer selection in rearing period therefore represent important tasks for every dairy operation. To support management decision, several support models were developed. The problem can be formulated as a multi-hierarchical Markov decision process or optimization dynamic programming model. The ability of farmers to make right decisions at the right times significantly determines the success of any enterprise. This success can be stated as maximizing profit. It has been shown that total profit is highly affected by reproductive performance (Britt, 1985).

Heifer replacement

Reproductive performance received special attention in the literature (Olynk and Wolf, 2009; Cabrera and Giordano, 2010; Giordano *et al*., 2011; Giordano *et al*., 2012, Ettema 2011) as a result of its prominent economic impact on the profitability of dairy operations. Numerous studies have analyzed the optimum replacement interval in dairy herds and factors that affect these decisions (van Arendonk, 1985; Kristensen, 1988; De Vries, 2004, Demeter *et al*., 2011; Cabrera, 2012). Simultaneous accounting of several biological and economic parameters is necessary to determine the optimum time of replacing a cow. Milk production level, pregnancy, stage of lactation, parity and transition probabilities such as involuntary culling, pregnancy, and abortion are considered the most important factors affecting replacement decisions (Kalantari *et al*., 2010). Alternative approaches have been proposed to handle these factors and find the optimum replacement strategy including marginal net revenue (MNR) (van Arendonk, 1984), dynamic programming (DP) (Smith, 1973; van Arendonk, 1985; De Vries, 2004), and stochastic simulation models (Kristensen and Thysen, 1991). The first two methods are based on the production function approach in which the cow's revenue and costs are modelled during cow's

lifetime (Groenendaal *et al*., 2004). The limitation of MNR is its inability to include the variation in expected milk production of the present cow and subsequent replacement heifers, and the genetic gain of replacement heifers (Groenendaal *et al*., 2004). The DP technique overcomes two limitations. However, due to its complexity, the usage of DP models has been restricted to research analysis and not for building decision support systems for practical decision-making and on-farm management. The Monte Carlo stochastic simulation approach has been used to calculate the total expected net returns during next year and that value was used for ranking animals. Kristensen and Thysen (1991) compared the decisions being made by DP and stochastic simulation and reported insignificant difference between the two models. Recently, Cabrera (2012) used a Markov chain simulation model to find a suboptimal replacement strategy. In brief, this method calculates the net present value for a cow and its potential replacement, which could be used to decide whether to keep or replace a dairy cow. This method does not have the complexity of DP models and overcomes the limitation of MNR method because it can include expected variations in the cow and replacement performances. Cabrera (2012) reported that trend and replacement strategies found with the newly Markov chain model would be similar to those found with DP models. However, such study did not include a formal comparison with a DP model. Kalantari *et al*. (2014) has found a strong correlation (95 %) in replacement decisions resulting from using two completely different modeling approaches: The classical and state-of the- art dynamic programming framework and a newly developed technique using simple simulation of Markov chains. Post optimality analyses demonstrated that overall long-term herd structure and herd net returns resulting from models' replacement policies were very similar. These results strongly support that the newly developed Markov chain is a good alternative for practical dairy decision-making and for the development of decision support systems.

Heifers rearing period

The replacement heifer program is particularly important, and its primary goal is to breed these animals at an early age with optimal body weight to achieve easy calving with minimum investments (Fricke, 2004). Dairy farmers face a complex dilemma in minimizing costs associated with rearing heifers while ensuring or enhancing lifetime economic productivity. Decisions about heifer management interact with underlying biological aspects of growth, thereby influencing future profitability of the herd (Mourits *et al*., 1999). 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). Dairy heifers are normally inseminated for the first time at about 15 months of age to calve at approximately 2-years of age. At the age of 15 months, they reach only about 60 % of mature body weight (Coffey *et al*., 2006). The management decision on when to start breeding is a management one, but it is generally influenced by nutrition and growth rate during the rearing period (Carson *et al*., 2002; Serjsen, 2005). Many studies suggest that the optimal AFC is ≤ 24 months (Mourits *et al*., 1999; Gabler and Heinrichs, 2003; Shamay *et al*., 2005). Pirlo *et al*., (2000) also confirmed that AFC can have a significant effect on both milk production capacity and longevity. The relation of lifetime milk performance to calves rearing period and fertility issues in the typical UK dairy farms was also evaluated by Wathes *et al*. (2008). However, most of those researchers based their conclusions on milk production rather than whole economic measurements. Ettema and Santos (2004) found that only 2.7 % of US Holstein dairy farms achieved the recommended targets of AFC ≤ 24 with live-weights ≥ 560 kg. The tendency for additional returns from higher number of new-born calves was also confirmed in sheep by Bonev and Kostadinova (2011). Fricke (2003, 2004) proved that the delay in age at first calving in heifers generated additional costs from higher culling rate, dystocia, and metabolic disturbances. According to the author, the optimum age at first calving of heifers was 24 months. Calving heifers at an older age has many disadvantages other than increasing their non-productive life and delaying potential milk income. When heifers calve at ages greater than 24 to 25 months, larger inventories or numbers of heifers must be maintained in the heifer herd. Increasing the age at calving also increases the generation interval, delaying the introduction of genetically superior replacements in the herd (Bailey *et al*., 2009).

Performance of Slovak Holstein dairy herds

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 Slovak Holstein herd had improved the length of productive life, number of lactations, lifetime yield as well as lifetime yield per day of Holstein dairy cows (BS SR, 2017). Based on the milk recording in control year 2014/2015 performed by the BS SR, it can be concluded that the lifetime milk yield of Holstein heifers first calving in 24 months amounted 21279 kg. The two additional months in AFC transformed in 104 € loss per cow. Adding another two months over the 26 months AFC will almost double the negative economic impact (-210 €). Holstein heifers first calving in 30 months have generated a -442.68 € loss a per cow basis. Negative impact on the lifetime yield was also well documented even for AFC below the optimal 24 months. Heifers with 21 months of AFC produced 1342 kg less amount of milk generating a -375.76 € loss in control year 2014/2015. Furthermore, the recent study by Huba *et al*. (2017) supports this results for Slovak Holstein dairy herds in 2016. The Holstein first calving heifers at 23 months 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 in 24 months.

Economic impacts

Mortality rates and culling of dairy animals are the critical indicators for dairy operation productivity. As reported by Fetrow *et al*. (2006) culling or exiting is the departure of cows from the herd because of sale, slaughter, salvage, or death. In most cases the cow that exits is replaced. The term "cull" than refers to all the cows that leave the dairy operation regardless of their destination or condition at departure. The report also implies that some may object to including cows that are sold for dairy purposes as part of a general cull category, as the word "cull" generally means to separate off for undesirable reasons. However, quantifying the amount of culling on dairies is highly beneficial in the comparison of herds (Fetrow *et al*., 2006). The literature review outlined a several approaches to describe the culling process on dairy farms including Terms like "yearly turnover" and "cows leave, %" (AgSource Cooperative Service, 2005), "culling rate" by Hoekema, 1999 a,b and also by Brett, 2003), "proportion removed from herd" (Smith *et al*., 2000), "percent left herd" (Gangwer *et al*., 1993), and "replacement rate" (Allaire, 1981). Dohoo and Dijkhuizen, 1993; Radke, 2000) even argued to distinguish the cause of culling as either "biological" (also known as "forced") or "economic". For any case mentioned, a more precise approach is to average the cow inventory at monthly intervals over the year (DRMS, 1997). Furthermore, Fertow *et al*. (2006) evaluated different herd turnover calculation methods. The calculation of herd turnover rate by two approaches for 4 combinations of herds, representing stable or expanding herds with moderate or intense culling. The alternative calculation (adding the number culled into the denominator) substantially underestimated the risk of culling in herds. The preferred calculation accurately estimates the risk of culling, even in rapidly expanding herds, as long as the mean cow inventory (denominator) was calculated on at least a monthly basis (Fertow *et al*., 2006). The pasture-based herd management system are to be reviewed with respect to the different routines and subsequent issues related to local conditions and husbandry systems used. This remark also supports the benchmarking with relevant peer operations and creation of individual farm information system. According to Compton *et al*. (2017) dairy industries and farmers need benchmarks for culling and mortality against which they can compare themselves, as well as improved understanding of the extent of any change and of any associated factors. Moreover, these events are inevitable and common, understanding their extent and causes at the herd or industry level is challenging because culling and mortality are influenced by economic, social, management, and animal disease factors. In turn, culling and mortality have important economic and animal welfare consequences (Compton *et al*, 2017). The study of de Vries *et al*. (2011) also indicated that high culling rates, including mortality, refers to the poor animal welfare status. Moreover, calf mortality has been identified as one of the most important indicators of dairy farm health status (Ortiz-Pelaez *et al*., 2008) and also represents economic losses to the dairy

industry due to delayed genetic progress, fewer replacements available for voluntary culling of the lactating herd, and increased cost of replacement (Raboisson *et al*, 2013). The annual economic damage resulting from stillborn and loss of calves is reported to be about $ 125 million (Meyer *et al*., 2001). It can be concluded that the total costs of calf and heifer mortality are probably underestimated (Ortiz-Pelaez *et al*., 2008). According to the work of Meyer *et al*. (2001), Berglund *et al*. (2003) and e.g. Steinbock *et al*. (2003), the continuous increase in calf-heifer mortality reported in many countries during the last decade suggests that the economic and welfare stakes related to the mortality of young cattle are also increasing. Furthermore, farmers also have been shown to alter their own culling criteria and decision making based on sociological issues (demographic characteristics, attitudes, education, degree of involvement in dairy groups) in addition to economic or biological ones (Beaudeau *et al*., 1996).

CONCLUSION

The management of dairy operations can be complex and daunting, while confronting many factors that are changing over time. Many farmers already discuss milk yields and other parameters among them, but sophisticated DSSs can provide more complex and independent analysis of dairy operation performance. The main goal of this approach is to improve individual profitability and better understanding of dairy business through evidence-based decisions.

There are a selection of tools available to perform on-farm analyses and assist in effective decision making process; but a key requirement is the system thinking element as a part of day-to-day farm routines rather than an ex-post evaluations. This remark also supports the benchmarking with relevant peer dairy farms and development of individual farm expert systems. Benchmarking tools typically help to identify where strengths and weaknesses lie within a farm business. By comparing with similar enterprises, benchmarking enables farmers to improve individual business performance and tackle the market volatility. This will ensure that the farm business is on the best possible roadmap for the future sustainable development.

Limitations of the uptake and lack of effective communication can be addressed by developing adapted DSS, using the multi-actor approach principles throughout the whole development process. By enabling dairy farmers to design and co-create potential solutions we may improve the implementation and speed up innovation on the ground through the interactive innovation model. It is important to incentivise scientists for their impact on agricultural practice by easy understandable outputs for end-users. The system of specific training courses will be needed to accomplish this task. Moreover, the availability of extension specialists or innovation transfer brokers for growing farming community would be limited in the future. Information and communication technologies have huge potential to partially tackle this issue with the switch from 'intuitive' to 'smart' decision making models. Effective adoption of specifically tailored DSS has already demonstrated the potential to bring economic, social and environmental benefits at local, national and global levels.

This study attempts to summarize the current knowledge about the logical basis of the decision support systems. The information provided here, however, may not be considered as complete. For instance, recent research aimed at national agricultural and innovation system structure and performance was not included in the present study. Further research is needed to better understand the links between the actors involved (e.g. farmers, advisors and scientists). The greater consideration of farm-level and end-user inputs and greater efficiency in respond to practice needs are critical.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Education, Science, Research and Sports of the Slovak Republic / the Slovak Research and Development Agency (project no. APVV-18-0121).

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.

AgSource Cooperative Service. (2005). Block G, yearly turnover. http:// www.crinet.com/hsblockg.htm.

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

Allaire, F. R. (1981). Economic consequences of replacing cows with genetically improved heifers. *Journal of Dairy Science*, 64, 1985−1995.

Alvarez, J. & Nuthall, 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, 48−60.

Andrew, M., Grundy, M., & Harris, C. (2013). *Decision support tools for agriculture*. http://www.csiro.au/en/Organisation-Structure/Flagships/Sustainable-Agriculture-Flagship/Decision-support-tools-agri.aspx. Accessed August 24, 2020.

Andrieu, N., Poix, C., Josien, E. & Duru, M. (2007). Simulation of forage management strategies considering farm-level diversity: example of dairy farms in the Auvergne. *Computers and Electronics in Agriculture*, 55, 36−48.

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. https://doi.org/10.1016/j.dss.2012.07.002

Bailey, T. & Currin, J. (2009). *Heifer Inventory and the Economics of Replacement Rearing*. https://www.pubs.ext.vt.edu/404/404-287/404-287.html. Accessed August 24, 2020

Barragan, A. A., Workman, J. D., BAS, S., Proudfoot, K. L. & Schuenemann, G. M. (2016): Assessment of an application for touchscreen devices to record calving-related events in dairy herds and monitor personnel performance. *Journal of Dairy Science*, 99, 662−5670. https://doi.org/10.3168/jds.2015-10841

Bayliss, H. R., Wilcox, A. & Randall, N. (2012). Does research information meet the needs of stakeholders? Exploring evidence selection in the global management of invasive species. *Evidence and Policy*, 8, 37−56.

Beaudeau, F., van der Ploeg, J. D., Boileau, B., Seegers, H. & Noordhuizen, J. P. T. M. (1996). Relationships between culling criteria in dairy herds and farmers' management styles. *Preventive Veterinary Medicine*, 25, 327−342.

Berglund, B., Steinbock, L. & Elvander, M. (2003). Causes of stillbirth and time of death in Swedish Holstein calves examined post mortem. *Acta Veterinaria Scandinavia*, 44, 111−120.

Bernet, T., Ortiz, O., Estrada, R. D., Quiroz, R. & Swinton, S. M. (2001). Tailoring agricultural extension to different production contexts: a user-friendly farm household model to improve decision-making for participatory research. *Agricultural Systems*, 69, 183−198.

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 business investment decisions. *Agricultural Finance Review*, 70, 97−125. https://doi.org/10.1108/00021461011042666

Binder, C. R., Feola, G., & Steinberger, J. K. (2010). Considering the normative, systemic and procedural dimensions in indicator-based sustainability assessments in agriculture. *Environmental Impact Assessment Review*, 30(2), 71−81. https://doi.org/10.1016/j.eiar.2009.06.002

Bond, A. J., & Morrison-Saunders, A. (2013). Challenges in determining the effectiveness of sustainability assessment. In A. J. Bond, A. Morrison-Saunders, & R. Howitt (Eds.), *Sustainability assessment: pluralism, practice and progress* (pp. 37−50). Routledge.

Bonev, G. & Kostadinova, N. (2011). Economic investigation on reproduction model in sheep farming. *Agricultural Economics and Management*, 56(6), 23−28.

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

Brett, J. (2003). What is the ideal culling rate? *Dairy Herd Manager*, 40, 100.

Britt, J. H. (1985). Enhanced reproduction and its economic implications. *Journal of Dairy Science*, 68, 1585−1592.

Brown, S. (1995). Measures of Perfection. *Sales and Marketing Management*, 104−105.

Bush, R. D., Windsor, P. A., Toribio, J. A. & Webster, S. R. (2008). Financial modelling of the potential cost of ovine Johne's disease and the benefit of vaccinating sheep flocks in southern New South Wales. *Australian Veterinary Journal*, 86, 398−403.

Cabrera, V. E., Breuer, N. E., Hildebrand, P. E. & Letson, D. (2005). The dynamic North Florida dairy farm model: a user-friendly computerized tool for increasing profits while minimizing N leaching under varying climatic conditions. *Computers and Electronics in Agriculture*, 49, 286−308.

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(1-4), 82−97. https://doi.org/10.1016/j.agee.2005.08.039

Cabrera, V. E. (2012). A simple formulation and solution to the replacement problem: A practical tool to assess the economic cow value, the value of a new pregnancy, and the cost of pregnancy loss. *Journal of Dairy Science*, 95, 4683−4698.

Cabrera, V. E. & Giordano, J. O. (2010). Economic decision making for reproduction. *Dairy Cattle Reproduction Conference*, St. Paul, MN, p. 77−86.

Calsamiglia, S., Astiz, S., Baucells, J. & Castillejos, L. (2018): A stochastic dynamic model of a dairy farm to evaluate the technical and economic performance under different scenarios. *Journal of Dairy Science*, 101, 7517−7530.

Carson, A. F., Dawson, L. E. R., McCoy, M. A., Kilpatrick, D. J. & Gordon, F. J. (2002). Effects of rearing regime on body size, reproductive performance and milk production during the first lactation in high genetic merit dairy herd replacements. *Animal Science*, 74, 553−565.

Castelan-Ortega, O. A., Fawcett, R. H., Arriaga-Jordan, C. & Herrero, M. (2003). A decision support system for smallholder campesino maize-cattle production systems of the Toluca Valley in Central Mexico. Part I – Integrating biological and socio-economic models into a holistic system. *Agricultural Systems*, 75, 1−21.

Cerf, M., Mathieu, A., Béguin, P. & Thiery, O. (2008). A collective analysis of co-design projects. Empowerment of the rural actors: a renewal of farming systems perspectives. *8th European IFSA Symposium*, pp. 1−6. Clermont-Ferrand, France.

Coffey, M. P., Hickey, J. & Brotherstone, S. (2006). Genetic aspects of growth of Holstein-Friesian dairy cows from birth to maturity. *Journal of Dairy Science*, 89, 322−329.

Compton, C. W. R., Heuer, C., Thomsen, P. T., Carpenter, T. E., Phyn, C. V. C. & McDougall, S. (2017). Invited review: A systematic literature review and meta-analysis of mortality and culling in dairy cattle. *Journal of Dairy Science*, 100, 1−16. https://doi.org/10.3168/jds.2016-11302

Coteur, I., Marchand, F., Debruyne, L., Daelemans, F. & Lauwers, L. (2016). A framework for guiding sustainability assessment and on-farm strategic decision making. *Environmental Impact Assessment Review*, 60, 16−23.

Council for Agriculture Science and Technology. (1999). Animal Agriculture and Global Food Supply, Task Force Report no. 135. Ames, Iowa, USA.

Cournut, S. & Dedieu, B. (2004). A discrete events simulation of flock dynamics: a management application to three lambings in two years. *Animal Research*, 53, 383−403.

Crosson, P., O'Kiely P., O'Mara, F. P. & Wallace, M. (2006). The development of a mathematical model to investigate Irish beef production systems. *Agricultural Systems*, 89, 349−370.

Dairy Records Management Systems. (1997). DHIA-202 herd summary, Fact Sheet A-1. Online. Available http://www.drms.org/PDF/materials/202fact1.pdf

Daniels, K. M. 2010. Dairy heifer mammary development. *Proc. 19th Annual Tri-State Dairy Nutrition Conference*, pp. 69–76, Ft. Wayne, IN.

De Jong. (2013). *Sustainable Dairy Production*. Wiley-Blackwell. ISBN: 978-0-470-65584-9

De Vries, M. & de Boer, I. J. M. (2009) Comparing environmental impacts for livestock products: a review of life cycle assessments. *Livestock Science*, 128, 1−11.

De Vries, M., Bokkers, E. A. M., Dijkstra, T., van Schaik, G. & de Boer, I. J. M. (2011). Invited review: Associations between variables of routine herd data and dairy cattle welfare indicators. *Journal of Dairy Science*, 94, 3213−3228. http://dx.doi.org/10.3168/jds.2011-4169.

Debauche, O., Mahmoudi, S., Andriamandroso, A. L. H., Manneback, P., Bindelle, J. & Lebeau, F. (2018): Cloud services integration for farm animals' behavior studies based on smartphones as activity sensors. *Journal of Ambient Intelligence and Humanized Computing*, 10(12), 4651−4662

Diaz-Solis, H., Kothmann, M. M., Hamilton, W. T. & Grant, W. E. (2003). A simple ecological sustainability simulator (SESS) for stocking rate management on semi-arid grazing lands. *Agricultural Systems*, 76, 655−680.

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

Dohoo, I. R. & Dijkhuizen, A. A. (1993). Techniques involved in making dairy cow culling decisions. *Compendium on Continuing Education for the Practicing Veterinarian*, 15, 515−520.

Eicker, S. W. & Fetrow, J. (2003). A prospective view of culling. *Midwest Dairy Herd Health Conference*. University of Wisconsin, Madison. https://nanopdf.com/download/a-prospective-view-of-culling-steve-eicker-dvm-ms_pdf

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.

El-Osta, H. S. & Morehart, M. J. (2000). Technology adoption and its impact on production performance of dairy operations. *Review of Agricultural Economics*, 22 (2), 477−498.

Ettema, J. F., Østergaard, S. & Sørensen M. K. (2011). Effect of including genetic progress in milk yield on evaluating the use of sexed semen and other reproduction strategies in a dairy herd. *Animal*, 5, 1887−1897.

Ettema, J. F. & J. E. Santos. (2004). Impact of age at calving on lactation, reproduction, health, and income in first-parity Holsteins on commercial farms. *Journal of Dairy Science*, 87, 2730−2742.

Fetrow, J., Nordlund, K. V. & Norman, H. D. (2006). Invited Review: Culling: Nomenclature, Definitions, and Recommendations. *Journal of Dairy Science*, 89, 1896−1905.

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

Franks, J. R. & Collis, J. (2003). *On-farm benchmarking: how to do it and how to do it better*. International Farm Management Association. 14th Congress, Perth, Western Australia, August 10-15.

Freebairn, D. M., Robinson, J. B. & Glanville, S. F. (2002). *Software tools for learning and decision support*. http://www.mssanz.org.au/MODSIM01/Vol%204/Freebairn.pdf

Fricke, P. M. (2004). Strategies for optimizing reproductive management of dairy heifers. *Proceedings of Western Canadian Dairy Seminar*, Red Deer, AB, Canada, 9-12 Mar 2004, pp. 163−176.

Fricke, P. M. (2003). Heifer reproduction. *Raising Dairy Replacements*. Midwest Plan Service. Ames, IA, pp.77−83.

Gabler, M. T. & Heinrichs, A. J. (2003). Dietary protein to metabolizable energy ratios on feed efficiency and structural growth of prepubertal Holstein heifers. *Journal of Dairy Science*, 86, 268−274.

Gangwer, M., Gamroth M. & Seldin, R. (1993). *10 important measures: Understanding dairy herd performance measurements from the Agri-Tech Analytics DHIA Herd Total Report, EM8540*. http://eesc.orst.edu/agcomwebfile/edmat/html/EM/EM8540/EM8540.html

Gargiulo, J. I., Eastwood, C. R., Garcia, S. C. & Lyons, N. A. (2018): Dairy farmers with larger herd sizes adopt more precision dairy technologies. *Journal of Dairy Science*, 101(6), 5466−5473.

Garmestani, A., Allen, C., Mittelstaedt, J., Stow, C., & Ward, W. (2006). Firm size diversity, functional richness, and resilience. *Environment and Development Economics*, 11(4), 533-551. https://doi.org/10.1017/S1355770X06003081

Gasparatos, A. & Scolobig, A. (2012). Choosing the most appropriate sustainability assessment tool. *Ecological Economics*, 80, 1−7. https://doi.org/10.1016/j.ecolecon.2012.05.005

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.

Gibson, R. B. (2006). Beyond the pillars: sustainability assessment as a framework for effective integration of social, economic and ecological considerations in significant decision-making. *Journal of Environmental Assessment Policy and Management*, 8, 259−280. https://doi.org/org/10.1142/S1464333206002517

Giordano, J. O., Fricke, P. M., Wiltbank, M. C. & Cabrera, V. E. (2011). An economic decision − making support system for selection of reproductive management programs on dairy farms. *Journal of Dairy Science*, 94, 6216−6232.

Giordano, J. O., Kalantari, A. S., Fricke, P. M., Wiltbank, M. C. & Cabrera, V. E. (2012). A daily herd Markov-chain model to study the reproductive and economic impact of reproductive programs combining timed artificial insemination and estrus detection. J*ournal of Dairy Science*, 95, 5442−5460.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M. & Toulmin, C. (2010) Food Security: The Challenge of Feeding 9 Billion People. *Science*, 327(5967) 812−818. https://doi.org/10.1126/science.1185383

Gómez-Limón, J. A., & Riesgo, L. (2009). Alternative approaches to the construction of a composite indicator of agricultural sustainability: An application to irrigated agriculture in the Duero basin in Spain. *Journal of Environmental Management*, 90(11),

3345−3362. https://doi.org/10.1016/j.jenvman.2009.05.023

Graham, R., Mancher, M., Miller Wolman, D., Greenfield, S. & Staiberg, E. (2011) *Clinical Practice Guidelines We Can Trust*, National Academic Press (US). https://doi.org/10.17226/13058

Groenendaal, H., Galligan, D. & Mulder, H. (2004). An economic spreadsheet model to determine optimal breeding and replacement decisions for dairy cattle. *Journal of Dairy Science*, 87, 2146−2157.

Hall, P., Holmes-Ling, P., Stewart, K. & Sheane, R. (2010). *A Scottish farm-based greenhouse gas accounting tool for Scotland.* Report for the Scottish Government by Laurence Gould Partnership Ltd. and Best Foot Forward.

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.

Henchion, M., Hayes, M., Mullen, A., Fenelon, M. & Tiwari, B. (2017). Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. *Foods*, 2017, 6(7), 53. https://doi.org/10.3390/foods6070053

Herve, D., Genin, D. & Migueis, J. (2002). A modelling approach for analysis of agro pastoral activity at the one-farm level. *Agricultural Systems*, 71, 187−206.

Hoekema, M. J. (1999a). *Guess what may be eating your lunch: The hidden costs of cull rate (part 1 of 2).* http://dairy.ifas.ufl.edu/DBAP/The%20hidden%20costs%20of%20cull%20rate%201

Hoekema, M. J. (1999b.) *Guess what may be eating your lunch: The hidden costs of cull rate (part 2 of 2).* http://dairy.ifas.ufl.edu/DBAP/The%20hidden%20costs%20of%20cull%20rate%202.pdf

Hopwood, B., Mellor, M., & Brien, G. O. (2005). Sustainable Development − Mapping Different Approaches. *Inter Science*, 52, 38−52. https://doi.org/10.1002/sd.244

Huba, J., Záhradník, M., Dianová, M. & Kotrbová, I. 2017. Dobrá správa – v našej populácii stúpa produkcia na deň života dojníc.. [Good news − the day production in our population per dairy cows' lives is rising..]. *Mini Info*, 10(4), 36−38.

Ingrand, S., Cournut, S., Dedieu, B. & Antheaume, F. (2003). Modelling beef herd management decisions. *INRA Productions Animales*, 16, 263−270.

Iribarren, D., Hospido, A., Moreira, M. T. & Feijoo, G. (2011). Benchmarking environmental and operational parameters through eco-efficiency criteria for dairy farms. *Science of the Total Environment*, 409(10), 1786−1798.

Jakeman, A. J. (Ed.), Voinov, A., Rizzoli, A. E. & Chen, S. H. (2008). Environmental modelling, software and decision support: state of the art and new perspectives: also as e-book. (*Developments in integrated environmental assessmen*t; Vol. 3). Amsterdam etc.: Elsevier.

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.

Kalantari, A. S., Mehrabani-Yeganeh, H. & Moradi, M. (2010). Determining the optimum replacement policy for Holstein dairy herds in Iran. *Journal of Dairy Science*, 93, 2262−2270.

Kalantari, A. S., Cabrera, V. E. & Solis D. (2014). A comparison analysis of two alternative dairy cattle replacement strategies: optimization versus simulation models. *Economía Agraria*, 18, 12−24.

Kaloxylos, A., Groumas, A., Sarris, V., Katsikas, L., Magdalinos, P., Antoniou, E., Politopoulou, Z., Wolfert, S., Brewster, C., Eigenmann, R. & Maestre Terol, C. (2014). A cloud-based Farm Management System: Architecture and implementation. *Computers and Electronics in Agriculture*, 100 168−179.

Kaspersson, E., Rabinowicz, E. & Schwaag Serger, S. (2002). EU milk policy after enlargement: competitiveness and politics in four candidate countries. Lund: Swedish Institute for Food and Agricultural Economics.

Korte, M., Lee, K. & Fung, C. C. (2012) Sustainability in Information Systems: Requirements and Emerging Technologies. In P*roc. 2012 International Conference on Innovation, Management and Technology Research (ICIMTR2012)*, Malacca, Malaysia, 21-22 May, 2012, pp. 481−485.

Kristensen, A. R. (1988). Hierarchic Markov processes and their applications in replacement models. *European Journal of Operational Research*, 35, 207−215.

Kristensen, A. R. & Thysen, I.1991. Ranking of dairy cows for replacement. *Acta Agriculturae Scandinavica*, 41, 295−303.

Krupová, Z., Michaličková, M. & Krupa, E. 2012. Review of methodologies for costs calculating of ruminants in Slovakia. *Journal of Central European Agriculture*, 2012, 13(3), 426−445. https://doi.org/10.5513/JCEA01/13.3.1068

Kustermann, B., Kainz, M. & Hulsbergen, K. J. (2008). Modeling carbon cycles and estimation of greenhouse gas emissions from organic and conventional farming systems. *Renewable Agriculture and Food Systems,* 23, 38−52.

Leeuwis, C., & Van den Ban, A. W. (2004). Communication for Rural Innovation: rethinking agricultural extension. Blackwell Publishing Ltd.Lehenbauer, T. W., and J. W. Oltjen. (1998). Dairy cow culling strategies: making economical culling decisions. *Journal of Dairy Science*, 81, 264−271.

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 2014. http://ifsa.boku.ac.at/cms/fileadmin/Proceeding2014/WS_1_1_Lindblom.pdf

Matzek, V., Covino, J. Funk, J. L. & Saunders, M. (2014) Closing the knowing-doing gap in invasive plant management: accessibility and interdisciplinarity of scientific research. *Conservation Letters*, 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. *U.S. Environmental Protection Agency Papers*, p. 71.

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

Meyer, C. L., Berger, P. J., Koehler, K. J., Thompson, J. R. & Sattler, C. G. (2001). Phenotypic trends in incidence of stillbirth for Holsteins in the United States. *Journal of Dairy Science*, 84, 515−523.

Mourits, M. C. M., Huirne, R. B. M., Dijkhuizen, A. A., Kristensen, A. R., Galligan, D. T. (1999). Economic optimization dairy heifer management decisions. *Agricultural Systems*, 61, 17−31. https://doi.org/10.1016/S0308-521X(99)00029-3

Murphy, S. P. & Lindsay, L. H. (2003). Nutritional importance of animal source foods. *Journal of Nutrition*, 133, 3932S-3935S.

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, 393−414.

Ness, B., Urbel-Piirsalu, E., Anderberg, S., & Olsson, L. (2007). Categorising tools for sustainability assessment. *Ecological Economics*, 60(3), 498−508. https://doi.org/10.1016/j.ecolecon.2006.07.023

Neumann, C., Harris, D. M. & Rogers, L. M. (2002). Contribution of animal source foods in improving diet quality and function in children in the developing world. *Nutrition Research*, 22, 193−220.

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' behaviour in strategic problem solving. In: *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.

Olynk, N. J. & Wolf, C. A. (2009). Stochastic economic analysis of dairy cattle artificial insemination reproductive management programs. *Journal of Dairy Science*, 92, 1290−1299.

Ortiz-Pelaez, A., Pritchard, D. G., Pfeiffer, D. U., Jones, E., Honeyman, P. and Mawdsley, J. J. (2008). Calf mortality as a welfare indicator on British cattle farms. *Veterinary Journal*, 176, 177−181.

Pacini, C., Wossink, A., Giesen, G. & Huirne, R. (2004). Ecological-economic modelling to support multi-objective policy making: a farming systems approach implemented for Tuscany. *Agriculture, Ecosystems & Environment*, 102, 349−364.

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. (1997). Improving the Uptake of Decision Support Systems in Agriculture. *Proceedings of First European Conference for Information Technology in Agriculture*, 129−134.

Perrings, C. (1998). Resilience in the dynamics of economy-environment system. *Environment and Resource Economics*, 11(3–4), 503−520.

Pirlo, G., Miglior, F. & Speroni, M. (2000). Effect of Age at First Calving on Production Traits and on Difference between Milk Yield Returns and Rearing Costs on Italian Holsteins. *Journal of Dairy Science*, 83, 603−608. http://dx.doi.org/10.3168/jds.S0022-0302(00)74919-8

Pla, L. M., Pomar, C. & Pomar J. (2003). A Markov decision sow model representing the productive lifespan of herd sows. *Agricultural Systems*, 76, 253−272.

Pope, J., & Morrison-Saunders, A. (2013). *Pluralism in practice. Sustainability assessment: pluralism, practice and progress* (pp. 100−114). Routledge.

Pope, J., Annandale, D., & Morrison-Saunders, A. (2004). Conceptualising sustainability assessment. *Environmental Impact Assessment Review*, 24(6), 595−616. https://doi.org/10.1016/j.eiar.2004.03.001

Poppe, K. J., Wolfert, S., Verdouw, C. & Verwaart, T. (2013). "Information and Communication Technology as a Driver for Change in Agri-Food Chains." *EuroChoices*, 12(1), 60−65.

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.

Radke, B. R. (2000). Production management: Sixteen dairy culling and replacement myths. *Compendium on Continuing Education for the Practising Veterinarian - North American Edition*, 22(2), S36−57.

Rajala-Schultz P. J. & Gröhn Y. T. (1999). Culling of dairy cows. Part III. Effects of diseases, pregnancy status and milk yield on culling in Finnish Ayrshire cows. *Preventive Veterinary Medicine*, 41, 295−309.

Randolph, T. F., Schelling, E., Grace, D., Nicholson, C. F., Leroy, J. L., Cole, D. C., Demment, M. W., Omore, A., Zinsstag, A. & Ruel, M. (2007). Invited review: Role of livestock in human nutrition and health for poverty reduction in developing countries. *Journal of Animal Science*, 85, 2788−2800.

Rennings, K., Wiggering, H. (1997). Step towards indicators of sustainable development: linking economic and ecological concepts. *Ecological Economics*, 20, 25−36.

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.

Rogers, Y., Sharp, H. & Preece, J. (2011). *Interaction Design: Beyond Human-Computer Interaction*. Hoboken, NJ: John Wiley.

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.

Rotz, C. A., Buckmaster, D. R. & Comerford, J. W. (2005). A beef herd model for simulating feed intake, animal performance, and manure excretion in farm systems. *Journal of Animal Science*, 83, 231−242.

Serjsen, K. (2005). *Mammary Development. Calf and heifer rearing: principles of rearing the modern dairy heifer from calf to calving* (ed. PC Garnsworthy), pp. 237−251. Nottingham University Press, Nottingham, UK.

Shamay, A., D. Werner, U. Moallem, H.Barash, & I. Bruckental. (2005). Effect of nursing management and skeletal size at weaning on puberty, skeletal growth rate, and milk production during first lactation of dairy heifers. *Journal of Dairy Science*, 88, 1460−1469.

Shepherd, M. & Wheeler, D. (2010). *OVERSEER® Nutrient Budgets – Thoughts on Developing a Decision Support Tool.* AgResearch Ltd. http://www.overseer.org.nz/LinkClick.aspx?fileticket=0ZZecZ-af7k%3D&tabid=81.

Smith, J. W., Ely, L. O. & Chapa, A. M. (2000). Effect of region, herd size, and milk production on reasons cows leave the herd. *Journal of Dairy Science*, 83, 2980−2987.

Smith, J., Sones, K., Grace, D., MacMillan, S., Tarawali, S. & Herrero, M., (2012). Beyond milk, meat, and eggs: Role of livestock in food and nutrition security. *Animal Frontier*s, 3, 6−13.

Steinbock, L., Nasholm, A., Berglund, B., Johansson, K. & Philipsson, J. (2003). Genetic effects on stillbirth

and calving difficulty in Swedish Holsteins at first and second calving. *Journal of Dairy Science*, 86, 2228−2235.

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

Stonehouse, D. P., Vos, G. W. D. & Weersink, A. (2002). Livestock manure systems for swine finishing enterprises. *Agricultural Systems*, 73, 279−296.

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. *7th International Conference on Electrical Engineering Computing Science and Automatic Control*, Chiapas, Mexico.

The Results of Dairy Herd Milk Recording in Slovak Republic. (2016). http://pssr.sk/wp-content/uploads/2016/12/Rocenka-Mlieko-2015-web.pdf

Turban, E., Aronson, J. E., Liang, J. E. & Sharda, R. (2007). *Decision support and business intelligence systems* (8th ed.). Upper Saddle River, New Jersey, USA: Pearson, Prentice Hall.

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? *Scandinavian Journal of Forest Research*. http://dx.doi.org/10.1080/02827581.2013. 837950

Van Arendonk, J. A. M. & Dijkhuizen, A. A. (1985). Studies on the replacement policies in dairy cattle. III. Influence of variation in reproduction and production. *Livestock Production Science*, 13, 333−349.

Van Arendonk. (1985). Studies on the replacement policies in dairy cattle. II. Optimum policy and influence of changes in production and prices. *Livestock Science*, 13(2), 101−121.

Van Calker, K. J., Berentsen, P. B. M., Giesen, G. W. J. & Huirne, R. B. M. (2005). Identifying and ranking attributes that determine sustainability in Dutch dairy farming. *Agriculture and Human Values*, 22, 53−63. https://doi.org/10.1007/s10460-004-7230-3

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.

Van Passel, S., & Meul, M. (2010). Multilevel sustainability assessment of farming systems: a practical approach. *Proceedings of the 9th European IFSA Symposium*, pp. 791−800.

Van Passel, S., Nevens, F., Mathijs, E., & Van Huylenbroeck, G. (2007). Measuring farm sustainability and explaining differences in sustainable efficiency. *Ecological Economics*, 62(1), 149−161. http://doi.org/10.1016/j.ecolecon.2006.06.008

Van Schaik, G., Nielen, M. & Dijkhuizen, A. A. (2001). An economic model for on farm decision support of management to prevent infectious disease introduction into dairy farms. *Preventive Veterinary Medicine*, 51, 289−305.

Veysset, P., Bebin, D. & Lherm, M. (2005). Adaptation to Agenda 2000 (CAP reform) and optimisation of the farming system of French suckler cattle farms in the Charolais area: a model-based study. *Agricultural Systems*, 83, 179−202.

Volk, M., Lautenbach, S., van Delden,. H, Newman, L. T. H. & 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.

Waas, T., Hugé, J., Verbruggen, A., & Wright, T. (2011). Sustainable Development: A Bird's Eye View. *Sustainability*, 3(12), 1637−1661. http://doi.org/10.3390/su3101637

Wathes, D. C., Brickell, J. S. Bourne, N. E., Swalia A. & Cheng, Z. (2008). Factors influencing heifer survival and fertility on commercial dairy farms. *Animal*, 2(8), 1135−1143 & *The Animal Consortium*. http://doi.org/10.1017/S1751731108002322

Weaver, P. M. & Rotmans, J. (2006). Integrated sustainability assessment: What is it, why do it and how? *International Journal of Innovation and Sustainable Development*, 1(4), 284−303.

Whittaker, C., McManus, M. & Smith, P. (2013). A comparison of carbon accounting tools for arable crops in the United Kingdom. *Environmental Modelling & Software*, pp. 1−12. http://dx.doi.org/10.1016/j.envsoft.2013.03.015.

Woodward, S. J. R., Romera, A. J., Beskow, W. B. & Lovatt, S. J. (2008). Better simulation modelling to support farming systems innovation: review and synthesis. *New Zealand Journal of Agricultural Research*, 51, 235−252.

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

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*. Nitra: SPU v Nitre, 131 p. http://c.?fn=detailBiblioForm&sid=FBA0A0C68B9B2F0CA2CF5BED718F

Záhradník, M., Huba, J., Brestenský, V. & Kumičík, M. (2018). EkonMOD milk – interaktívny model farmy dojníc [EkonMOD milk – interactive model of a dairy farm]. *AGROmagazín*, 20(5), 30–31.

Záhradník, M. & Huba, J. (2018). Vek jalovíc pri prvom otelení je ekonomicky významným ukazovateľom, jeho optimalizáciu podporuje platforma EkonMOD milk [Heifers age at first calving is an important economic indicator, its optimimisation is supported by the EkonMOD milk tool]. *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, 1002-1008. ISBN 978-80-552-1503-7. (Book of Abstracts: ISBN 978-80-552-1500-6, p. 146). 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, 1009-1013. ISBN 978-80-552-1503-7. (Book of Abstracts: ISBN 978-80-552-1500-6, s. 147). http://spu.fem.uniag.sk/mvd2016/proceedings/en/articles/s12/zahradnik_pokrivcak2.pdf

Záhradník, M., Pokrivčák. J. & Tóth, M. (2018). Modelling the milk production and economics in a dairy farm. In: *International Scientific Days 2018: "Towards Productive, Sustainable and Resilient Global Agriculture and Food Systems"*: Nitra, SR, 16.-17. 5. 2018: Book of Conference Proceedings. Nitra. ISBN 978-80-552-1503-7

Zhong-xiao, L., & Yimit, H. (2008). Environmental decision support system development for soil salinization in the arid area oasis. *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, September, 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 up-to-date instructions for authors are available on the journal website: https://www.sjas.ojs.sk

Slovak Journal of Animal Science

Subscription and distribution

All orders should be sent direct to:

Slovak Journal of Animal Science
Editorial Office
NPPC – Research Institute for Animal Production Nitra
Hlohovecká 2
951 41 Lužianky, Slovak Republic

Phone: +421 37 6546 249
E-mail: sjas@nppc.sk

Subscription rates 2020 (journal + postage)

Print:
65,- € collecting in editorial office directly
75,- € Slovak Republic
85,- € Czech Republic
95,- € Europe
105,- € ROW

Offprints:
Editorial office will send on request the offprint in Portable Document Format (PDF) via e-mail.

Slovak Journal of Animal Science, 53, 2020 (3)

Contents

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