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Contents

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Slovak J. Anim. Sci., 52, 2019 (2): 47–52

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STAUROSPORINE-INDUCED APOPTOSIS: ANALYSIS BY DIFFERENT ANNEXIN V ASSAYS

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ABSTRACT

Annexin V assay is a well-known method for the evaluation of cell apoptosis. However, there is a wide range of available kits at the market that are of different quality and price. In this study, staurosporine-induced apoptosis model was used in order to compare low-cost and high-cost Annexin V detection kits from three different producers. Briefly, two human cell lines (KG-1 and NKT) were treated with staurosporine for 1, 3 and 6 hours and then evaluated for the apoptosis induction using 3 different detection kits by flow cytometry. Both cell lines showed significant ($P < 0.05$) increase in cell apoptosis after 3 hours (20 % and 13 %, respectively) and 6 hours (50 % and 20 %, respectively) of incubation with staurosporine. No significant changes in the proportion of apoptotic cells were observed by comparing the data detected using low-cost or high-cost Annexin V assays. In conclusion, tested low-cost Annexin V detection kits could be safely used for an objective and fast flow cytometric assessment of the apoptosis in human or animal cells. However, the efficiency of these kits for the evaluation of apoptosis for example by immunofluorescence microscopy in other cells or specific tissue e.g. embryos could differed. As fluorescence microscopy is less sensitive than flow cytometry, this technique should require an Annexin V detection kit of higher quality that does not have to agree with our findings for flow cytometry.

Key words: cell culture; apoptosis; staurosporine; annexin V assay; flow cytometry

INTRODUCTION

Apoptosis is one of the most fundamental biological processes in mammals that occurs normally during development and aging and maintain homeostasis in multicellular organisms. Apoptosis also occurs as a defence mechanism such as in immune reactions or when cells are damaged by disease or noxious agents (Norbury and Hickson, 2001). Although there are a wide variety of stimuli and conditions, both physiological and pathological, that can trigger apoptosis, not all cells will necessarily die in response to the same stimulus (Elmore, 2007). Apoptosis is typically accompanied by the activation of a class of caspases and widespread biochemical and morphological changes to the cell (Nicholson *et al*., 1997; Porter *et al*., 1997). An important biochemical feature is the expression of cell surface markers that result in the early phagocytic recognition of apoptotic cells by adjacent cells, permitting quick phagocytosis with minimal compromise to the surrounding tissue. This is achieved by the movement of the normal inward-facing phosphatidylserine (PS) of the cell's lipid bilayer to expression on the outer layers of the plasma membrane (Bratton *et al*., 1997).

Annexin V is a recombinant phosphatidylserine--binding protein that interacts strongly and specifically with phosphatidylserine residues and can be used

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for the detection of apoptosis (Van Engeland *et al*., 1998; Arur *et al*., 2003). Annexin V based assay is already known for more than two decades and can be applied both in flow cytometry and in fluorescent microscopy. Koopman *et al*. (1994) were the first to describe a method using extrinsically applied hapten (i.e. FITC or biotin) labeled nnexin V to detect apoptosis. Labeled annexin V binds in the presence of Ca^{2+} to PS residues that are exposed at the outer leaflet of the plasma membrane of apoptotic cells. Annexin V is not able to bind to normal vital cells since the molecule is not able to penetrate the phospholipid bilayer. In dead cells, however, the inner leaflet of the membrane is available for binding of extrinsically applied annexin V, since the integrity of the plasma membrane is lost. To discriminate between dead and apoptotic cells, a membrane impermeable DNA stain, such as propidium iodide (PI) can be added simultaneously to the cell suspension. In this way vital, apoptotic and dead cells can be discriminated on basis of a double-labeling for annexin V and PI, and analyzed by flow cytometry (Van Engeland *et al*., 1998).

Staurosporine is a microbial alkaloid, isolated from *Streptomyces* sp. cultures. Staurosporine has been shown to: inhibit cell cycle progression in a variety of cell lines (Abe *et al*., 1991; Crissman *et al*., 1991; Bruno *et al*., 1992); enhance differentiation of human promyelocytic leukemia cells (Okazaki *et al*., 1988; Okuda *et al*., 1991); inhibit tumor cell invasion (Schwartz *et al*., 1990); and induce morphological changes typical of apoptosis in rat cardiomyocytes (Yue *et al*., 1998) and rat hippocampal neurons (Krohn *et al*., 1998).

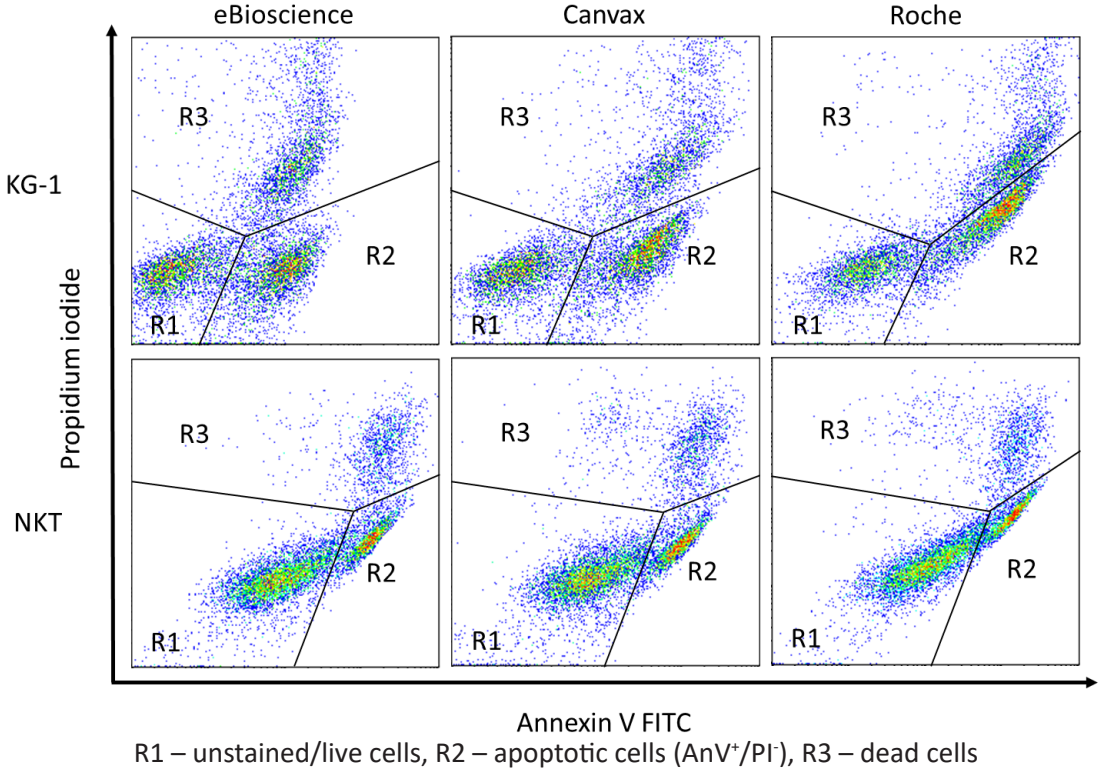

Figure 1. Illustrative dot plots showing evaluation strategy of the proportion of apoptotic cells within the control and staurosporine-induced cells

Many important mechanisms involved in apoptosis have been demonstrated in staurosporine- induced apoptosis models (Xia *et al*., 1995; Jacobsen *et al*., 1996).

At the present there are many Annexin V assay kits available at the market. These kits usually differ in their quality and price. The aim of this study was therefore to compare the quality of Annexin V assay kits of different costs from three different producers by measuring of apoptotic cells using staurosporine-induced apoptosis model and flow cytometry.

MATERIAL AND METHODS

In this study, two types of human cell lines were used in the experiments: non-adherent KG-1 cell line and adherent stromal NK.tert cell line (NKT). Both cell lines were generously provided by Dr. Medhat Shehata (Medical University of Vienna, Austria). Briefly, both cell lines were treated with staurosporine (Santa Cruz Biotechnology, USA) at the concentration of 1 µM for $5x10^5$ cell/ml in specific culture medium: a) in RPMI-1640 medium with 10 % FBS and 1 % antibiotics for KG-1 cells or b) αMEM culture medium (both media from Thermo Fisher Scientific, USA) with 20 % FBS and 1 % antibiotics for NKT cells. Cells were cultured in T25 culture flasks at 37 °C and 5 % CO_2 for 1, 3 and 6 hours. For each time interval the control flasks (cells not treated with staurosporine) were also incubated. The experiment was replicated for three times.

After each time incubation, cells were harvested by centrifugation (KG-1) or using 0.05 % Trypsin-EDTA (NKT) and stained according to the very similar manufacturer's protocols with Annexin V assays obtained from three different producers: Annexin V-FITC Apoptosis Detection Kit (low-cost; Canvax, Spain), Annexin V-FITC Apoptosis Detection Kit (low-cost; eBioscience, Thermo Fisher Scientific, USA) and Annexin-V-FLUOS Staining Kit (high-cost; Roche Slovakia, Slovak Republic). Cells were incubated also with propidium iodide (Molecular Probes, Switzerland) in order to detect dead cells and analysed using a FACSCalibur flow cytometer (BD Biosciences, USA) with cell populations gated to distinguish apoptotic (AnV^+/PI^-) cells (Figure 1). At least 10,000 cells were analysed in each sample.

Obtained results were evaluated using the SigmaPlot software (Systat Software Inc., Germany) with one-way ANOVA (Holm-Sidak method) and expressed as the means ± SEM.

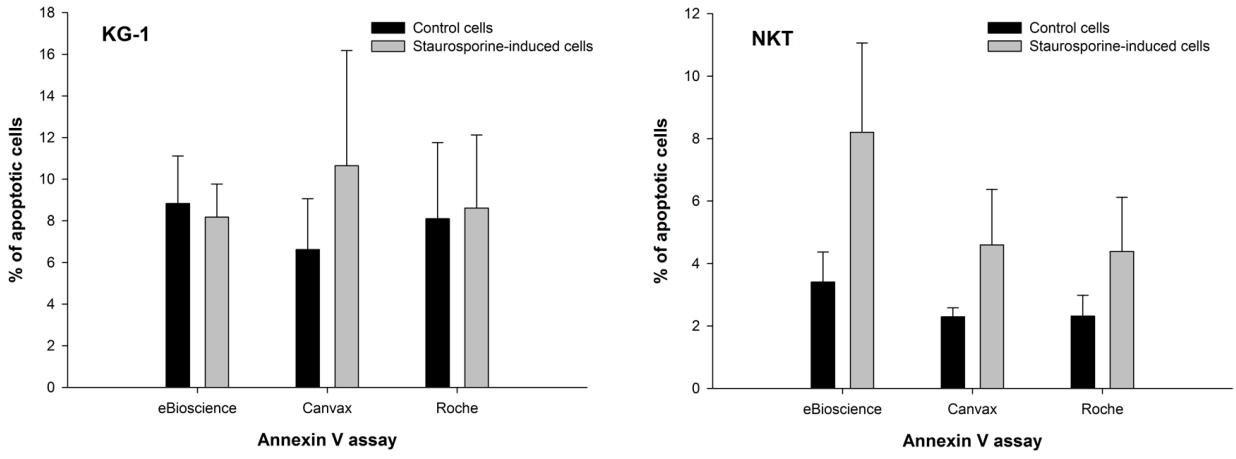

Figure 2. Proportion of apoptotic cells in control and staurosporine-induced cell lines after 1 hour of incubation

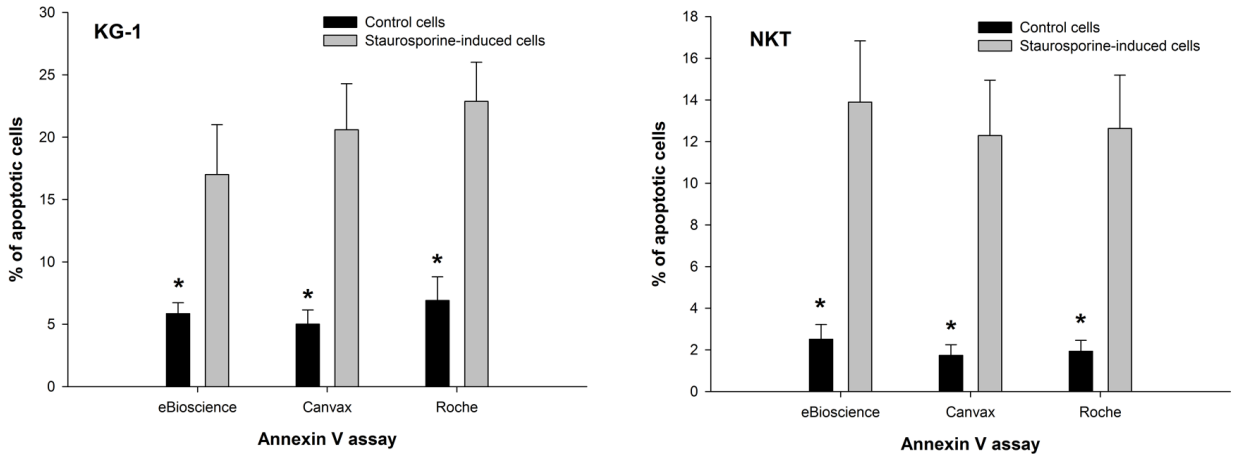

Figure 3. Proportion of apoptotic cells in control and staurosporine-induced cell lines after 3 hours of incubation

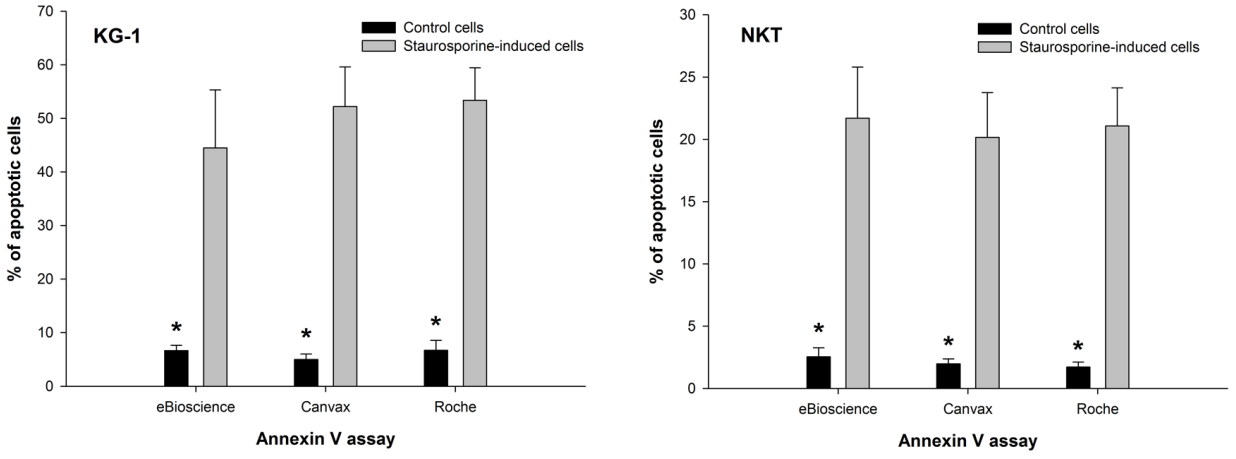

Figure 4. Proportion of apoptotic cells in control and staurosporine-induced cell lines after 6 hours of incubation

RESULTS AND DISCUSSION

In the presented study, cells were incubated with staurosporine for 1, 3 and 6 hours. Flow cytometry did not reveal significant apoptosis induction in KG-1 (about 8 %) or NKT cell line (about 4 % of apoptotic cells) after 1 hour of incubation using any of tested Annexin V assays in comparison to control (uninduced) cells (Figure 2). On the other hand, after 3 hours of incubation staurosporine significantly ($P < 0.05$) induced apoptosis in both cell lines in comparison to control cells (about 20 % in KG-1 and 13% in NKT; Figure 3). However, no significant differences were observed among the used Annexin V assays. The most significant ($P < 0.05$) induction of apoptosis by staurosporine was observed in both cell lines after 6 hours of incubation (about 50 % in KG-1 and 20 % in NKT; Figure 4). Similarly, no significant differences in the percentage of detected apoptotic cells were noticed among the used low-cost or high-cost Annexin V assays.

Obtained results indicated that the efficiency of staurosporine-induced apoptosis in cells strongly depends on the cell type as well as on the incubation time (2-fold increase in the proportion of apoptotic cells when compared KG-1 to NKT cell line and even when compared 3 h to 6 h of incubation; Figure 3 and 4). Antonsson and Persson (2009) observed about 40 % of apoptotic cells using Annexin V assay in non-adherent human leukemic cell line U-937 treated with staurosporine for 24 hours. Belmokhtar *et al*. (2001) noticed distinct ability of staurosporine to trigger apoptosis even in the two different sublines of the same non-adherent cell line L1210 (L1210/S and L1210/0). About 60 % of apoptotic cells were detected in both sublines via Annexin V assay after 3 hours and 12 hours, respectively. Concerning the adherent cells, staurosporine was an effective apoptosis inducer of porcine aortic endothelial cells as determined by Annexin V assay (Kabir *et al*., 2002). In this study, staurosporine treatment for 1 h increased the proportion of apoptotic cells to 33 % of the total cell population as compared with 7 % in control untreated cells. Moreover, after 24 h of incubation with staurosporine the percentage of apoptotic cells increased to 90-95 %. Thus, those studies support our findings about the different effect of staurosporine according to the treatment period and type of treated cells.

CONCLUSION

Staurosporine successfully induced apoptosis in both types of human cell line with different induction efficiency according to the type of cells (adherent or non-adherent) or the time of the incubation. Moreover, this staurosporine-induced apoptosis model definitely proved that the quality of tested low-cost and high-cost Annexin V assays were comparable when used for flow cytometry. At least, the same model could be used for animal cell lines in various biological experiments for an objective and fast assessment of apoptotic cells.

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MORPHOLOGICAL CHARACTERISTICS OF DONKEYS (*EQUUS ASINUS*) IN KABYLIE AREA, ALGERIA

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ABSTRACT

The survey was to define some morphometric characteristics and body biometric indexes of donkeys sampled in the Kabylie area, Algeria. The study was carried out from February to June 2018 in Bejaia and Tizi-Ouzou province. The study population included 124 males and 2 females. In total, 17 body measures were selected for morphometric characterization including and seven body biometric indexes were calculated. Body weight estimated the two equations was 144.3 ± 23.9 and 171.5 ± 28.8 kg, respectively. Significant higher body weight was recorded in the age group ≤ 5 years and the lower body weight in the age group ≥ 6 - ≤ 10 years and ≥ 11 years. Morphological variables of chest width (CW) and Cannon length (CL) were significant longer ($P < 0.02$) in aged donkeys (25.2 ± 1.3 and 20.5 ± 0.7 cm, respectively) compared to adult donkeys (24.7 ± 2.3 and 20 ± 1.4 cm, respectively). Aged donkeys (114.8 ± 5.8 cm) were also significantly superior ($P < 0.01$) concerning the thoracic circumference (TC) compared to adult donkeys (112.2 ± 9.8 cm). The highest values were found between WH and BH ($r = 0.80$); HR and BH ($r = 0.72$) HR and WH ($r = 0.72$) ($P < 0.05$). Dactyl thoracic Index (DTI), Compact Index (CI), Massive index (MI) and Relative body index (RBI) appeared to be influenced by donkey ages ($P > 0.05$). This is a first report on the phenotypic characterization in donkeys in Kabylie area (Algeria) based on corporal measurements. Our comparative analysis of morphometric parameters; such as back length, body length, neck length; suggests that donkeys of Kabylie area are typically invariant among breeds and it has not been changed through the periods.

Key words: donkeys; morphometric characterization; Kabylie; Algeria

INTRODUCTION

Donkey (*Equus asinus*) is an odd-toed ungulate and the smallest species in the Equidae family (Grinder *et al*., 2006). Donkeys in their nature are very friendly, calm, quite, patient, intelligent, cautious, playful, and eager to learn and enjoy the company of humans. It is characteristically short-legged with exceptionally long ears. Importance of donkeys is also conferred through their use in riding tourism and as eco-friendly means of pack and transportation when compared with horses (GOVS, 2005). Donkeys (*Equus asinus*) represent an important component of Algerian livestock and make a significant contribution to the agricultural economy; serving as draft animals.

According to the year 2001 inventory; the donkeys population is estimated 180160 heads in Algeria (FAO, 2003) found essentially in the northern regions, where they are particularly appropriate to tolerate the hard conditions of works. As draught animals, donkeys play a major role in the economy of developing countries by being the main source in transport and traction, particulary in areas with difficult reliefs. However, despite the donkey's popularity, information regarding various morphological characteristics in this species is limited (Labbaci *et al*., 2018).

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The capacity performance of donkeys could be assessed by the description of the morphological characteristics, such as umbilical girths, body length and height. This has been suggested as donkey draft power is directly proportional to size parameters (Nengomasha, 1999). In donkey, during domestication, some morphological and genetic changes have taken place in order to survive better in given conditions (Rossel *et al*., 2008). In African continent, the typical factors (high daily temperatures, minimal amount of precipitation and lack of nutriment quality) enabled donkeys to develop typical aptitudes, which played a key role to survive in dry areas (Pearson and Ouassat, 2000). The knowledge of morphometric measurements in donkey is of great importance for the genetic diversity preservation and development and taxonomic affiliation. Thus, the general objective of the current study was to contribute to a better knowledge of donkey in Algeria, especially in Kabylie region, known for its typical mountains. The survey was to define some morphometric characteristics and body biometric indexes of donkeys sampled in Bejaia and Tizi-Ouzou province. The correlation coefficients different between body measurements were estimated.

MATERIAL AND METHODS

Area study

The study was carried out from February to June 2018 in the Kabylie area, Algeria. Different localities of Bejaia (36° 43' N, 5° 04' E) and Tizi-Ouzou (36° 42' N, 4° 2' E) province were chosen randomly. The topography of Kabylie area is mostly predominated by mountainous. The vegetation is mainly composed of several species of trees and natural or cultivated herbs. Constitute part of climate is Mediterranean region. The maximum summer temperature are ranged from 30.3 to 36.3 °C (July) and the minimum winter temperature are ranged from 6.6 to 6.7 °C (February).

Animal and measurements

The study population included 124 males and 2 females. The donkeys are divided in 3 age groups namely ≤ 5 (young), ≥ 5 - ≤ 10 (adulte), ≥ 11 (aged). In total, 17 body measures were selected for morphometric characterization including. Linear measures (Figure 1) as head length (HL), ear length (EL), neck length (NL), chest width (CW), back length (BaL), body length (BoL), hips width (HW), umbilical circumference (UC), back height (BH), height at the rump (HR), thoracic circumference (TC), chest depth (CD), withers Height (WH), front leg length (FLL), cannon circumference (CC), cannon length (CL), cannon height (CH) were performed using a specially graduated measuring tape. The ages of donkeys were determined from the donkey owners and controlled by dentition analysis (Daveze and Raveneau, 2002). The identification of robe color was performed by direct observation under natural daylight and the frequency distribution of each phenotype was estimated.

From some measured morphometric donkeys, seven body biometric indexes were calculated according to the following formulas. Body Profile Index (BPI) = WH/BoL (Mariante *et al*., 2002); > 0.90: long and good animal for speed; 0.86 - 0.88: medium conformation animal or < 0.85: small conformation animal, fit for traction. Pectoral height index (PHI) = CD/FLL (Marcenac *et al*., 1980); 0.50 ≤ PHI ≤ 0.55: leggy animal or PHI > 0.56: leg shorted. Dactyl thoracic index (DTI) = CC/TC (Chabchoub *et al*., 2004); this index define three animal types: hypermetric, eumetric and elliptical. Compact index (CI) = BW/WH (Boujenane *et al*., 2008). Front-back height in (FBH) = WH/HR (Marcenac *et al*., 1980); FBH ≤ 1: straight back (no overload) or FBH > 1: the anterior region is higher than the posterior (overload). Massive index (MI) = TC/WH (Mariante *et al*., 2002); MI ≤ 1: support well its weight or MI > 1: massive overload. Relative Body Index (RBI) = BoL/TC (Nicks *et al*., 2006); RBI ≥ 0.90: longilinear, 0.84 ≤ RBI ≤ 0.89: mediolinear or RBI ≤ 0.83: brevilinear.

The body weight (BW) for each animal was calculated according to two validated formulas:
BW-1 = $TC^{2.65}/2188$ (Pearson and Ouassat, 1996) or BW-2= $(WH^{0.24})$ x $(TC^{2.576})$ x 0.000252 (Eley and French, 1993).

Statistical analysis

Data were analyzed using a mixed model for repeated measurements (Statview Software, Version 4.55) taking into account an autocorrelation between data obtained successively on the same animal. The data (± SD) were expressed as values of the donkey body measurements (cm). The animal

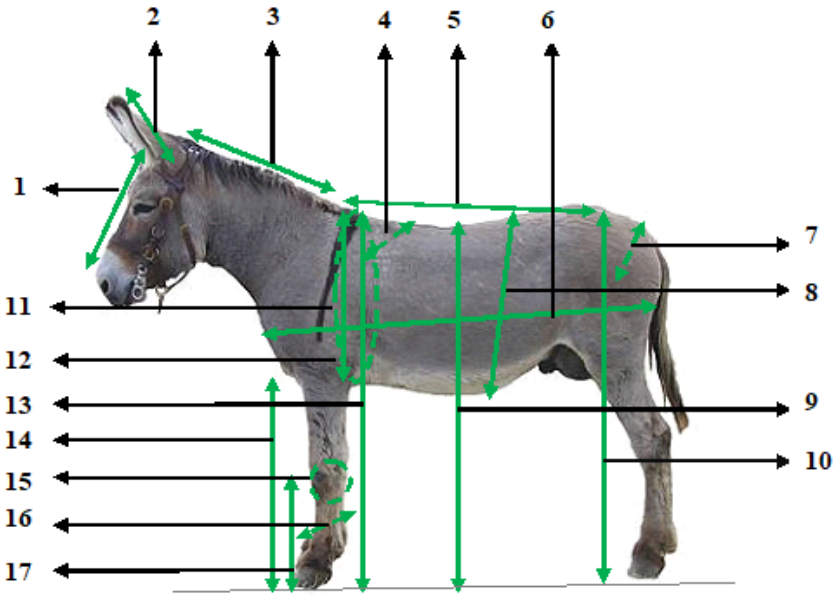
weight and donkey body measurements were analyzed using age (young: ≤ 5 years aged; adult: ≥ 5 - ≤ 10 years aged; aged: ≥ 11 years) as factors of variation. The one way variance analysis (ANOVA) was used to evaluate the obtained data. The values were statistically different when the *P*-value was < 0.05.

RESULTS

The frequency of the coat color (Figure 2) showed that 59.5 % of the donkeys had various shades of brown, 27 % grey and 13.5 % black. The body weight according to the age of donkeys is illustrated in Figure 4. Body weight estimated the two equations was 144.3 ± 23.9 and 171.5 ± 28.8 kg, respectively. The higher body weight was recorded in the age group ≤ 5 years and the lower body weight in the age group ≥ 6 - ≤ 10 years and ≥ 11 years (Figure 3). A significant difference of body weight was observed between the young donkey group and the aged donkey group (P < 0.05).

Descriptive statistics of morphological variables including mean, standard deviation, minimal-maximal and coefficient of variation are depicted in Table 1. Mean values of morphological variables and their standard for each age group are shown in Table 2. Morphological variables of chest width (CW) and Cannon length (CL) were significant longer (P < 0.02) in aged donkeys (25.2 ± 1.3 and 20.5 ± 0.7 cm, respectively) compared to adult donkeys (24.7 ± 2.3 and 20 ± 1.4 cm, respectively). Aged donkeys (114.8 ± 5.8 cm) were also significantly superior (P < 0.01) concerning the thoracic circumference (TC) compared to adult donkeys (112.2 ± 9.8 cm).

Phenotypic correlation coefficients (r) among morphologic variables and body weight are given in Table 3. The highest values were found between WH and BH (r = 0.80); HR and BH (r = 0.72) HR and WH (r = 0.72) (P < 0.05). Other high values were found between CW and BLL (r = 0.60), WH and CH (r = 0.56), WH and HR (r = 0.56) (P < 0.05). The correlation values of TC-CH, WH-CH, WH-HR, HL-TC, BaL-WH, BoL-WH and TC-WH presented values



1 - Head length (HL); 2 - Ear length (EL); 3 - Neck length (NL); 4 - Chest width (CW); 5 - Back length (BaL); 6 - Body length (BoL); 7 - Hips width (HW); 8 - Umbilical circumference (UC); 9 - Back height (BH); 10 - Height at the rump (HR); 11 - Thoracic circumference (TC); 12 - Chest depth (CD); 13 - Withers Height (WH); 14 - Front leg length (FLL) 15 - Cannon circumference (CC); 16 - Cannon length (CL); 17 - Cannon height (CH)

Figure 1. The different body measurements performed in donkey

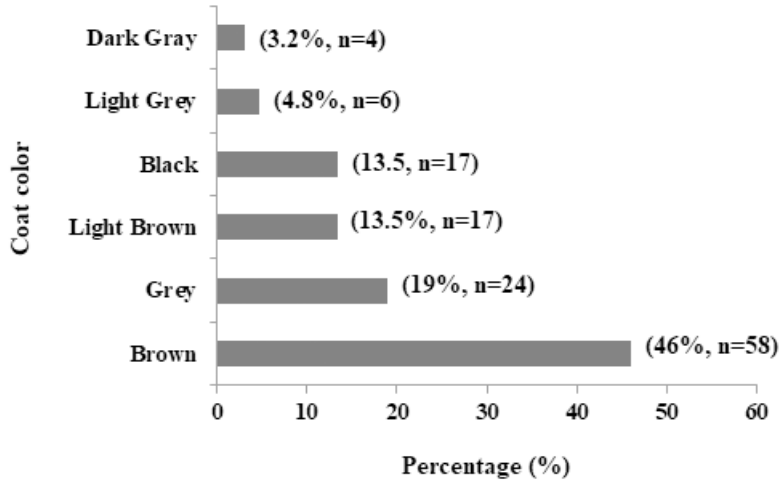
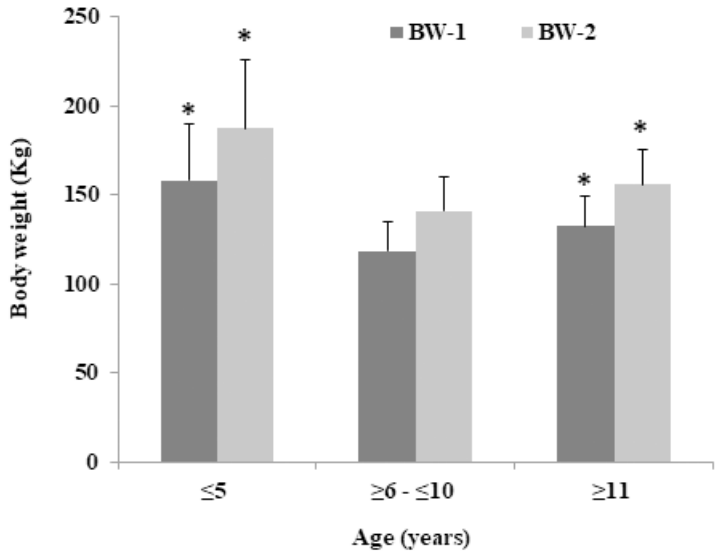


Figure 2. Frequency distribution of coat color of donkey in Kabylie area, Algeria

ranged between 0.51 and 0.58 ($P < 0.05$). Other low or very low correlation values were found between the others morphological parameters. There were also no high negative correlations between all other traits. For the body weight, the correlations were more marked with TC ($r = 0.99$), moderately marked with HL; WH and CH ($0.50 \geq r \leq 0.70$), and weakly marked with the rest of the morphological parameters ($P < 0.05$).

The results of body biometric indexes are summarized in Table 4. Dactyl thoracic Index (DTI), Compact Index (CI), Massive index (MI) and relative body index (RBI) appeared to be influenced by donkey ages ($P > 0.05$). The averages of the DTI, CI, MI and RBI index are 0.18 ± 0.01, 1.34 ± 0.2, 1.11 ± 0.06 and 0.93 ± 0.06, respectively. The BPI, FBH and PHI indexes are 0.97 ± 0.05; 0.66 ± 0.04 and 0.98 ± 0.04 respectively.



Means with the same superscripts in each weight of different ages are significantly different (*$P < 0.05$).

Figure 3. Weight of donkey by age groups (young: ≤ 5 years aged; adulte: ≥ 5 - ≤ 10 years aged; aged: ≥ 11 years)

Table 1. Descriptive analysis of donkey body measurements in Kabylie area, Algeria

	Mean ± SD	Min-Max	Median	CV (%)	95 % CI
BW-1 (kg)	144.3 ± 23.9	91.2 - 107.5	146.2	0.165	473 - 718.1
BW-2 (kg)	171.5 ± 28.8	107.5 - 250.8	172.8	0.168	679.7 - 1031.9
HL (cm)	48.5 ± 3.3	40 - 56	48	0.069	9.1 - 13.8
EL (cm)	24.4 ± 1.8	20 - 28	25	0.074	2.7 - 4.1
NL (cm)	46 ± 4.7	33 - 56	47	0.102	18.1 - 27.4
CW (cm)	25.6 ± 1.9	20 - 29	26	0.073	7.1 - 10.8
BaL (cm)	63.2 ± 2.5	58 - 72	63	0.039	5 - 7.6
BoL (cm)	110.1 ± 5.9	91 - 130	110	0.054	28.6 - 43.4
HW (cm)	32.4 ± 1.6	29 - 40	32	0.051	2.2 - 3.4
UC (cm)	141.1 ± 10.3	108 - 161	142	0.073	86.3 - 131
BH (cm)	107.2 ± 5.3	92 - 120	107.5	0.039	23.2 - 35.2
HR (cm)	109.6 ± 4.8	97 - 118	110	0.044	18.9 - 28.7
TC (cm)	118.5 ± 7.5	100 - 137	119.5	0.063	45.9 - 69.8
CD (cm)	49.2 ± 1.94	44 - 56	49	0.039	3.1 - 4.7
WH (cm)	106.9 ± 5.4	94 - 118	107	0.051	24.3 - 36.9
FLL (cm)	75 ± 3.9	51 - 82	75	0.052	12.6 - 19.2
CC (cm)	14.7 ± 1.1	12 - 23	15	0.078	1.1 - 1.6
CL (cm)	21.07 ± 1.72	14 - 25	21	0.081	2.4 - 3.7
HC (cm)	31.98 ± 2.92	17.5 - 38	32	0.091	7 - 10.6

Min: minimal value; Max: maximal value; CV: coefficient of variation; CI: confidence interval.

Table 2. Morphometric measurements of the donkeys in Kabylie area, Algeria

Body variables (cm)	Young donkeys (≤ 5 years) (n = 13) (Mean ± SD)	Adult donkeys (≥ 6 - ≤ 10 years) (n = 62) (Mean ± SD)	Aged donkeys (≥ 11 years) (n = 51) (Mean ± SD)
HL	47.8 ± 5.3^{a}	46.3 ± 2.3^{a}	47.1 ± 2.1
EL	24.8 ± 1.9	23.6 ± 2.4	23.7 ± 1.6
NL	44.4 ± 5.4	48.9 ± 1	48.2 ± 1.6
CW	25.6 ± 2.1^{a}	24.7 ± 2.3^{a,b}	25.2 ± 1.3^{b}
BaL	63.5 ± 3.8^{a}	61.7 ± 1.5^{b}	62.1 ± 2.2^{a,b}
BoL	107.3 ± 8.7^{a}	109.6 ± 3.5	108.4 ± 6.4^{a}
HW	33.2 ± 2.9^{a,b}	32.5 ± 0.9^{a}	32.1 ± 2^{b}
UC	137.5 ± 14.6	142.8 ± 12.5	143.4 ± 9.3
BH	105.8 ± 6	107.8 ± 5.3^{a}	105.8 ± 4.2^{a}
HR	110.2 ± 5.8	108.7 ± 5.3^{a}	107.2 ± 3.7^{a}
TC	112.2 ± 9.8^{a,b}	110.2 ± 5.9^{a}	114.8 ± 5.8^{b}
CD	48.8 ± 3.2^{a,b}	49.6 ± 1.2^{a}	48.4 ± 2.1^{b}
WH	106 ± 6.3	105.3 ± 4.8	103.2 ± 5.2
FLL	73.5 ± 7.5^{a}	77.2 ± 1.6^{a,b}	73.5 ± 3.2^{b}
CC	14.5 ± 1.3^{a}	14.8 ± 0.6^{a,b}	14.7 ± 0.9^{b}
CL	21.5 ± 1.6	20 ± 1.4	20.5 ± 0.7
CH	32.5 ± 2.1^{a}	30.3 ± 0.6^{a,b}	30.8 ± 1.1^{b}

^{a,b} Means with the same letters superscripts in each row of different ages are significantly different (P < 0.05).

Table 3. Phenotypic correlation coefficients (r) between body measurements in donkeys (*P < 0.05)

	BW-1	BW-2	HL	EL	NL	CW	BaL	BoL	HW	UC	BH	HR	TC	CD	WH	FLL	CC	CL	CH
BW-1																			
BW-2	**0.99***																		
HL	**0.53***	**0.55***																	
EL	033*	0.34*	0.23*																
NL	-0.08	-0.08	-0.35*	0.07															
CW	0.40*	0.40*	-0.01	0.12	0.13														
BaL	0.48*	0.50*	0.43*	0.28*	-0.08	0.34*													
BoL	0.34*	0.37*	0.27*	0.47*	0.14	0.05	0.29*												
HW	0.29*	0.30*	0.15	0.31*	0.02*	0.23	0.32*	0.25*											
UC	0.17	0.19*	0.32*	0.30*	-0.08	-0.13	0.26*	0.33*	0.22*										
BH	0.36*	0.40*	0.36*	0.38*	0.05	0.09	0.41*	0.46*	0.40*	0.46*									
HR	0.49*	**0.52***	0.32*	0.40*	-0.04	0.08	0.41*	0.49*	0.36*	0.44*	**0.72***								
TC	**0.99***	**0.99***	**0.54***	0.33*	-0.07	0.40*	0.45*	0.35*	0.26*	0.17	0.36*	0.47*							
CD	0.37*	0.39*	0.22*	0.16	-0.04	0.39*	0.47*	0.25*	0.27*	0.22*	0.40*	0.31*	0.36*						
WH	**0.51***	**0.56***	0.45	0.41*	-0.09	0.20	**0.53***	**0.51***	0.41*	0.36*	**0.80***	**0.72***	**0.51***	0.45*					
FLL	0.25*	0.26*	0.07	0.21*	0.22*	**0.60***	0.40*	0.28*	0.39*	0.13	0.40*	0.39*	0.24*	0.35*	0.41*				
CC	0.25*	0.25*	0.19*	0.01	0.14	0.12	0.16	0.10	0.16	0.19*	0.08	0.14	0.25	0.17	0.14	0.19*			
CL	0.39*	0.40*	0.39*	0.36*	-0.06	0.17	0.32*	0.35*	0.18	0.18	0.35*	0.42*	0.39*	0.29*	0.41*	0.29	0.16		
CH	**0.58***	**0.60***	0.25*	0.37*	-0.03	0.33*	0.38*	0.32*	0.34*	0.19*	0.43*	**0.56***	**0.58***	0.31*	**0.56***	0.36*	0.16	0.46*	

Head length (HL); Ear length (EL); Neck length (NL); Chest width (CW); Back length (BaL); Body length (BoL); Hips width (HW); Umbilical circumference (UC); Back height (BH); Height at the rump (HR); Thoracic circumference (TC); Chest depth (CD); Withers Height (WH); Front leg length (FLL); Cannon circumference (CC); Cannon length (CL); Cannon height (CH).
* P < 0.05

Table 4. Morphometric index of the donkeys in Kabylie area, Algeria

Index	Young donkeys (≤ 5 years) (n = 13) (Mean ± SD)	Adult donkeys (> 6 - < 10 years) (n = 62) (Mean ± SD)	Aged donkeys (> 11 years) (n = 51) (Mean ± SD)	Donkeys Total (n = 126) (Mean ± SD)
BPI	0.99 ± 0.05	0.97 ± 0.05	0.97 ± 0.05	0.97 ± 0.05
PHI	0.67 ± 0.08	0.66 ± 0.03	0.66 ± 0.04	0.66 ± 0.04
DTI	0.18 ± 0.01[a]	0.17 ± 0.02[b]	0.18 ± 0.01[a,b]	0.18 ± 0.01
CI	1.46 ± 0.26[a]	1.36 ± 0.2	1.3 ± 0.16[a]	1.34 ± 0.2
FBH	0.96 ± 0.03	0.98 ± 0.04	0.98 ± 0.04	0.98 ± 0.04
MI	1.14 ± 0.05[a,b]	1.11 ± 0.06[a]	1.09 ± 0.07[b]	1.11 ± 0.06
RBI	0.89 ± 0.04[a,b]	0.93 ± 0.07[a]	0.95 ± 0.06[b]	0.93 ± 0.06

Body Profile Index (BPI), Pectoral height index (PHI), Dactyl thoracic index (DTI), Compact index (CI), Front-back height (FBH), Massive index (MI), Relative Body Index (RBI).
[a,b] Means with the same letter superscripts in each row of different ages are significantly different (P < 0.05).

The analysis of the correlation coefficients between the biometric indexes (Table 5) shows both negative and positive correlations (P < 0.001). Particularly significant positive correlation (P < 0.001) is recorded between CI and MI (r = 0.816) on one hand with significant negative correlation (P < 0.001) between CI and RBI (r = -0.71) on the other hand.

Table 5. Correlation coefficients (r) between morphometric index in donkeys

	BPI	PHI	DTI	CI	FBH	MI	RBI
BPI	-	-	-	-	-	-	-
PHI	0.038	-	-	-	-	-	-
DTI	-0.095	-0.065	-	-	-	-	-
CI	-0.001	0.034	-0.464*	-	-	-	-
FBH	0.395*	0.045	-0.011	-0.046	-	-	-
MI	0.233*	0.085	-0.469*	**0.816***	-0.329*	-	-
RBI	**-0.536***	-0.104	0.474*	**-0.71**	-0.006	**-0.69***	-

Body Profile Index (BPI), Pectoral height index (PHI), Dactyl thoracic index (DTI), Compact index (CI), Front-back height in (FBH), Massive index (MI), Relative Body Index (RBI). *P < 0.001.

DISCUSSION

Around world, and particularly in Africa, donkey is suitable in difficult regions, especially in mountainous area. They played a major role in the evolution of agriculture until the introduction mechanization that neglected this animal. Traditionally, donkeys are part of the Algerian agricultural systems used as a mean of products transport and animal draft, especially in Kabylie area. The morphobiometric characterization has been proposed as one of the strategies for analyzing and characterization of domestic populations (Bouchel *et al.*, 1997). The general objective of the current study was therefore to evaluate the morphometric variation and some biometric indexes; and to estimate the correlation coefficient between measurements of donkey in Kabylie region.

Out of a total of 126 donkeys, only two females were sampled in the present study. In Kabylie region, as in all of North Africa, donkey is certainly the most used animal in the daily life of people, especially in the village constructions. However, there are no donkey females in Kabylie area. Indeed, possession of a donkey female is not allowed for traditional reasons as breeding are located in the other regions of Algeria.

The results of survey revealed that the coat color was diversified with a predominance of brown color (46 %) following by grey color (19 %). In another survey conducted in the Tlemcen area of the East Northern Algeria, Labbaci *et al.*, (2018) reported a similar observation with the presence of five different classes of color of the studied donkeys. In Bulgaria, the coat color also varies where the more common colors are brown (57 %) and grey (Vleava *et al.*, 2016). The body coat color frequencies the Turkish donkeys are: mouse gray, white, black and brown (31.4 %, 24.7 %, 23.7 % and 20.2 %, respectively) (Yimlez and Ertuğrul, 2012). In Ethiopia, a variability of coat color in donkeys has been reported from some country localities (Kefena *et al.*, 2011). In North African region, there are two fundamental denominations of the donkey "*aγyul*" and "*ayzeḍ*", very widespread in Berber language. The word "aγyul" could be a term related to the brown color and derivative of the verb "*iγwal*" which means to be brown in the Touareg population of Southern Algeria (Camps *et al.*, 1985). Our results show that the donkey population was heterogeneous in Kabylie region. This difference of coat color could be attributed to ecological patterns and altitudinal gradients (Gizaw *et al.*, 2007).

Body weights were compared between young, adult and aged donkeys. Our results corroborate with those reported by Ebangi and Vall (1998) revealing a consistent development in body weight for estimated from 1 to 8 years with a decline thereafter. A similar donkey body weight was found in south-western Zimbabwe (Nengomasha *et al.*, 1996; 1999). In another study, the body weight average was higher than those reported by Nininahazwe *et al.* (2017) in West African and Stanišić *et al.* (2015) in Serbia. Also, this body weight is lower compared to investigation in Morocco (Boudjenane *et al.*, 2008). The differences between the average weight values can be

explained by the condition of the donkeys when taking the measurements, but also by the formulas used to estimate body weight.

Our findings revealed that young animals, adults, and aged animals do not present the same body parameters and this increases concomitantly with age for certain parameters (Table 3). There was a significant difference between the values of some variables measurements (CW, TC and CL) according to the animal age classes. This would be due to the fact that the physiological evolution according to animal age leads to an increase in weight and morphological growth. These findings corroborate with results obtained previously (Roamba, 2014; Kaboré, 2014; Nininahazwe *et al.*, 2017; Labbaci *et al.*, 2018). The size and body dimensions of donkeys in Kabylie region were similar to those reported in other parts of Africa e.g. Morocco (Pearson and Ouassat, 1996), Zimbabwe (Nengomasha *et al.*, 1999). It is reported by Wilson (1981) that there is little physical variation in donkeys found throughout Africa. Algerian donkeys have a less long body length than donkeys of Bulgaria (Barzev, 2004), Cyprus (Barzev, 2004), Turkey (Yilmaz and Ertuğru, 2012) and Martina Franca (Barzev, 2004). In Kabylie region, donkeys are used for pack transport to carry all types of merchandise e.g. during the olive picking period. The results of this study noticed that donkeys are small in size compared to the other mentioned above. This can be explained by the difficulty of living conditions such as food quantity and quality and work intensity.

From the analysis of obtained results, the correlations among 17 morphological variables observed, in general, are positive ($P < 0.05$) and similar to those reported in numerous studies (Folch and Jordana, 1997; Yilmaz and Ertuğru, 2012; Yilmaz *et al.*, 2013; Daloum *et al.*, 2015; Sobotková *et al.*, 2015).

The correlations between BW and some measurements were significant ($p < 0.05$). Regardless of the age of the donkey, the TC was the only measure highly correlated with the both results of BW formulas. Many investigations have reported a correlation coefficient of 0.90 between BW and TC (Pearson and Ouassat, 1996; Nengomasha *et al.*, 1999; Hassan *et al.*, 2013; Nininahazwe *et al.*, 2017). Furthermore, Aluja et al (2005) confirmed that the thoracic circumference was found to be an easier and more reliable measurement compared to the umbilical circumference which could be affected by other factors such as the moment of food ingestion, the food quantity and the physiological state (gestation).

In order to study deeply donkey conformation in Kabylie area, some indexes were assessed from the morphometric measurements. Our results have shown a statistically conclusive difference of biometric indexes (DTI, CI, MI and RBI) between different age groups. It is difficult to compare these results with others reported in literature because of the lack of studies on biometric indexes in donkeys.

The body profile index was 0.97. This value allows to classify the animal population as a longlinear breed ($BPI < 1$), meaning that its total length is substantially equal to its height. These results corroborate with those reported in donkey by Daloum *et al.* (2015) and, Folch and Jordana (1997) but seem to disagree with the results obtained in the Arabian horses Barbe and Barbe (Chabchoub *et al.*, 2004). The dactylo thoracic index shows a relationship between the mass of individuals and the members that sustain it. The DTI of the donkeys studied is defined as animals among to the category of hypermetric donkeys ($DTI < 1$). These results are comparable to those obtained in Spain (Folch and Jordana, 1997), Tchad (Daloum *et al.*, 2015) and Cameroun (Defeu *et al.*, 2015).

The compact index explains that the body mass of the studied donkey is greater than its size, i.e. the animal does not support its weight. In this study, the donkeys sampled have massive overload (1.34 ± 0.2 kg/cm), this corroborate with those reported by Daloum *et al.* (2015) and Defeu *et al.* (2015). The MI confirmed that donkeys studied in Kabylie area have a body overload ($MI > 1$). Similar characteristic is found in domestic donkeys of the Sahelian region, Tchad (Daloum *et al.*, 2015). Moreover, there was a high positive correlation between CI and MI ($r = 0.816$, $P < 0.001$).

The pectoral height index (PHI) indicated that donkeys are short-legged. The front-to-back height (FBH) suggests that donkeys have a posterior region higher than the anterior region. In this current investigation, it is revealed that donkeys are short-legged ($PHI > 0.56$) with a straight back ($FBH \leq 1$). Our finding does not corroborate with those reported by Folch and Jordana (1997), where the height at the withers and the height at the rump are equal in the Catalan race, in other terms well

balanced. The massive index indicates whether the animal supports its weight.

According to the relative body index (RBI), the obtained results (0.93 ± 0.06) confirm that donkeys are elongated in the study area (RBI > 0.90). Recently, Defeu *et al.* (2015) recorded a high RBI in the domestic donkeys of Northwest Cameroon. However, a low of RBI values has been found in different Algerian horse breed (Guedaoura *et al.*, 2011). A negative correlation was found between CI and RBI ($r = -0.71$, $P < 0.001$), i.e. the weight varies with the body length. A considerable genetic variability was observed between our results and cited studies previously. This difference could be attributed to the geographical origin of donkey genetics, which adapts to the warm and humid environment that affects the growth and development of the body.

CONCLUSION

This is a first report on the phenotypic characterization in donkeys in Kabylie area (Algeria) based on corporal measurements. Our comparative analysis of morphometric parameters; such as back length, body length, neck length; suggests that donkeys of Kabylie area are typically invariant among breeds and it has not been changed through the periods. These obtained results constitute a baseline data for a deeper understanding of the genetic diversity in equines and for using in genetic improvement. However, the molecular characterization would better identify donkey breeds in Algeria.

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EFFECT OF SORGHUM GRAIN INCLUSION IN MONTBELIARDE DAIRY COWS DIET ON HEALTH STATUS

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ABSTRACT

The present study investigated the effect of sorghum grain inclusion in dairy cows diet, as an alternative to barley grain, on plasma biochemical markers (glucose, triglycerides, cholesterol, total protein, albumin, urea, creatinine, total bilirubin, calcium, phosphorus, magnesium, iron, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, lactate dehydrogenase, creatine kinase). Twelve Montbeliarde dairy cows, mid to late lactation, averaging 698 ± 27 kg body weight and 18 ± 1.3 l milk.day^{-1}, were divided into two groups in a 43-day feeding trial: C diet, based on classical energetic sources (corn and barley) and E diet, where sorghum grain replace barley. The cows received the same forage diet. An analyzer BS-130 was used to determine the plasma parameters. Results showed that the use of 25 % sorghum grain in E diet increased (+30 %, P = 0.009) the plasma glucose and decreased the triglycerides (-20 %, P = 0.032) comparing to C diet. Total protein and albumin increased (+10 %, P = 0.002; respectively +17 %, P = 0.013) as effect of dietary sorghum addition. The calcium concentration increased (+21 %, P = 0.022), while the magnesium concentration decreased (-28 %, P = 0.010) in E diet compared to C diet. The enzymes profile slightly increased as effect of fed sorghum grain, but the differences between treatments were not significant (P > 0.05). Replacement of barley grain with sorghum had no adverse effects on health status of dairy cattle, the assessed markers being within the health reference limits.

Key words: dairy cows; barley; sorghum grain; health status; plasma profile

INTRODUCTION

In ruminant nutrition, cereal grains comprise up to 95 % of total diet (McCuistion, 2014). In the future, the use of cereals in grain distilleries and ethanol production, associate with climate change consequences, will increased interest in the utilization of alternative energy sources for the ruminant's nutrition (Gibreel *et al.*, 2009). Thereby, alternative valuable nutritional sources must be evaluated in order to partially or totally replace the classical cereal grains used in dairy cows feeding (Rattray, 2012). Sorghum grain (*Sorghum Vulgare* L.) is considered an important crop for both human and animal nutrition (Dicko *et al.*, 2006) and it was recommended as a suitable energetically resource for ruminants (Brouk and Bean, 2012; Mavromicalis, 2014; Khajehdizaj *et al.*, 2014; Yahaghi *et al.*, 2012). Nowadays, new improved sorghum varieties characterized by a high drought-tolerant capacity, a high yield production, nutritive value close to that of corn and a low tannin content, are available on the market. Blood metabolites are important indicators for animal health, the function of certain tissues and organs and, also provide important information about the effects of different nutritional regimen used in animals feeding. There is still a lack of published information on the effects

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of feeding sorghum grain as substitute of barley on dairy cows performance and health status (Ishler, 2017; Nikkhah *et al.*, 2004; Yahaghi *et al.*, 2012), except few research study reported in fattening steers (Voicu *et al.*, 2014; Voicu *et al.*, 2016). Therefore, the aim of this study was to evaluate the effect of sorghum grain as replacement of barley in dairy cows diets on some plasma metabolites related with health status.

MATERIAL AND METHODS

Dairy cows were treated in accordance with Romanian law no. 305/2006 regarding handling and protection of animals used for experimental purposes. All experimental procedures were approved by the Ethical Committee of the National Research-Development Institute for Animal Biology and Nutrition, Balotesti, Romania.

Animals, feeding and housing

Twelve multiparous Montbeliarde dairy cows, mid to late lactation, averaging 698 ± 27 kg body weight, 175 ± 10 days in milk (DIM), parity number 2.86 ± 0.60 and an initial milk yield of 18 ± 1.3 kg.day^{-1}, were used in a trial of 43 days. The trial design consisted of a 14 day adaptation followed by 29 day experimental period for sample collection. The animals were assigned to two homogenous groups and fed with a control diet (C) based on corn, barley, wheat bran and sunflower meal and an experimental diet (E), where the 25 % sorghum grains replace barley in the compound feed. The bulk feed (fed *ad libitum*) consisted of spring hay (60 % oat hay + 40 % vetch hay) and alfalfa haylage. The bulk to concentrate ratio was 60:40. Feed was given in two allowance per day at 06h and 16h. Diets were adequate to the category of weight and level of production and provided the following nutritional intakes: 18.0 kg dry matter (DM)/cow/day, 17.0 milk feed units (mFU)/cow/day and 1600 g intestinally digestible protein (IDP)/cow/day. During the 43 days trial period, the cows were housed in a conventional shelter equipped with collective feeding boxes and free access to feed and water.

Measurements, analyses and statistics

Standardized methods, as per Commission Regulation (EC) no. 152 (2009), were used to determine the gross chemical composition of the feed ingredients, compound feeds and of the bulk feed. The chemical composition and the nutritive values of the compound feed (CFs) are shown in Table 1.

Table 1. Chemical composition and nutritive value of the compound feed for dairy cows (g.kg DM^{-1})

Item	C	E
Analyzed and calculated values		
Dry matter	882	876
Organic matter	941	944
Gross energy (MJ)	18.95	18.71
Crude protein	143	131
Ether extract	23	23
Crude fiber	84	73
Nitrogen-free extractives	692	717
Ash	59	56
Nutritive values		
mFU	1.16	1.17
IDPN	98	91
IDPE	101	101
Ca	9.5	9.75
P	6.2	6.0

C, control diet (barley grain); E, experimental diet (sorghum grain); DM, dry matter; mFU, milk feed units; IDPN, intestinal digestible protein derived from nitrogen; IDPE, intestinal digestible protein derived from energy; Ca, calcium; P, phosphorus.

The diets were formulated according to the system adopted in Romania by Burlacu *et al.* (1991, 2002), based on the French model of evaluation (INRA, 1988).

The health state of the animals was monitored and accurately reflected in the blood constituents. At the end of experimental period (43 days), after the first milking in the morning before feeding blood samples were aseptically collected by jugular venepuncture into heparinized Vacutainer tubes (Vacutest®, Arzergrande, Italy), from all dairy cows (N = 12). Blood samples were immediately placed on ice, and 9000 µL of each sample was centrifuged for 25 minutes at 3500 x g. Concentration of glucose, cholesterol, triglycerides, total protein, albumin, urea, creatinine, total bilirubine, calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe) and the activity of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (AP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH) and creatine kinase (CK) were determined from blood plasma (4500 – 5000 µL) on an automatic BS-130 Chemistry analyzer (Bio-Medical Electronics Co., LTD, China), using standardized kits ACCENT 200, supplied by PZ Cormay S.A. Poland (Ţăranu *et al.*, 2014). Results are expressed as mean with standard error of the mean (SEM). Statistical differences between groups for different parameter concentrations were determined using SPSS – general linear model (Statistics version 20, 2011). The significance of differences between groups were estabilished using analysis of variance and Tukey's test. Differences between mean values were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

In the current study, replacement of barley with sorghum grain in the CFs of dairy cows did not affected ($P > 0.05$) the total dry matter intake (18.30 DMI kg.day^{-1}, C diet *vs.* 18.61 DMI kg.day^{-1}, E diet) or the consumption of the bulk feed, that was relatively similar between groups (20.82 DMI kg/head/day, C diet *vs*. 21.03 DMI kg/head/day, E diet), this revealed that the inclusion of sorghum grains did not affect the palatability of the ration. Furthermore the digestive processes were not affected by dietary treatments (e.g. absence of diarrhoea or other disorders). However, the sorghum diet had significant influence ($P < 0.05$) on the milk production (18.06 L.d^{-1}, C diet *vs*. 19.73 L.d^{-1}, E diet; data not shown).

The results of plasma biochemical parameters are summarized in Table 2.

Plasma energy profile

Plasma energy parameters ranged between physiological limits for the species and category (Merk Veterinary Manual, 2010). It is well known that glucose is essential for all organisms (Aschenbach *et al.*, 2010) and cereal grains are important sources that can provide non-fibrous carbohydrate (Van Knegsel *et al.*, 2007). Ruminal and intestinal fermentation of non-fibrous carbohydrate (particularly starch) release propionate, a glucose precursor for tissue and milk synthesis and theoretically increases substrate available for gluconeogenesis (Taylor and Allen, 2005). Van Knegsel *et al.* (2007) have demonstrated that plasma glucose concentrations increased when ruminants were fed with high amounts of energy sources.

In our study, the use of 25 % sorghum grain in E diet, as a substitute of barley grain, increased significant (+30 %, $P = 0.009$) the plasma glucose. The results agree with previous study (Aguerre *et al.*, 2009, Yahaghi *et al.*, 2012) which reported that the plasma glucose concentration was greater when cattle and sheep were fed with a fresh temperate pasture supplemented with sorghum grain (15 g.kg^{-1} of their body weight) compared to non-supplemented animals. Also, Nikkhah *et al.* (2004) noticed an increased glucose level of Holstein cows in mid-lactation stage in response to 21 days feeding of 20 % ground sorghum. Recently, Ishler (2017) reported that increased concentrations of plasma glucose level in sorghum diet *vs.* barley diet is surprisingly since the rumen digestion of barley is higher than that of sorghum. This effect could be attributed to a lower rumen degradability of sorghum grain non-fibrous carbohydrate which encourages a relatively high amount of starch entering into the small intestine and to the fact that enzymatic hydrolysis activities provides energy into the blood stream, in the form of glucose. The plasma triglycerides concentration significantly decreased (20 %, $P = 0.032$) as effect of feeding cow E diet compared with C diet. The plasma cholesterol concentration of cow fed E diet was insignificantly increase (+29 %, $P = 0.283$),

Table 2. Effect of feeding sorghum grain in dairy cows on plasma metabolic parameters

Plasma profile	Parameter	Limits*	C diet	E diet	SEM	*p-value*
Energy	Glucose, mg.dL^{-1}	40 – 100	42.03^{b}	54.58^{a}	3.21	0.009
	Triglycerides, mg.dL^{-1}	–	27.01^{a}	22.50^{b}	1.14	0.032
	Cholesterol, mg.dL^{-1}	62 – 193	88.61	113.88	15.51	0.283
Protein	Total protein, g.dL^{-1}	6.7 – 7.5	6.76^{b}	7.46^{a}	0.28	0.002
	Albumin, g.dL^{-1}	2.5 – 3.8	3.00^{b}	3.50^{a}	0.17	0.013
	Urea, mg.dL^{-1}	10 – 25	18.40	14.96	2.06	0.267
	Creatinine, mg.dL^{-1}	0.5 – 2.2	1.32	1.43	0.16	0.790
	Total bilirubin, mg.dL^{-1}	0 – 1.6	0.17	0.17	0.02	0.996
Mineral	Calcium, mg.dL^{-1}	8 – 11.4	8.37^{b}	10.14^{a}	0.53	0.022
	Phosphorus, mg.dL^{-1}	5.6 – 8.0	3.24	4.27	0.46	0.113
	Magnesium, mg.dL^{-1}	1.5 – 2.9	2.20^{a}	1.72^{b}	0.07	0.010
	Iron, µg.dL^{-1}	–	100.79	128.36	15.01	0.212
Enzyme	Alanine aminotransferase, U/L	6.9 – 35	33.35	35.96	6.01	0.235
	Aspartate aminotransferase, U/L	60 – 125	61.97	67.46	7.94	0.215
	Alkaline phosphatase, U/L	18 – 153	35.33	42.30	8.72	0.713
	Gamma-glutamyl transferase, U/L	6 – 17.4	15.93	17.24	2.70	0.218
	Lactate dehydrogenase, U/L	309 – 938	900.25	990.46	57.60	0.414
	Creatine kinase, U/L	0 – 350	168.80	173.04	12.16	0.143

C, control diet (barley grain); E, experimental diet (sorghum grain); Alanine aminotransferase, ALAT; Aspartate aminotransferase, ASAT; Alkaline phosphatase, AP; Gamma-glutamyl transferase, GGT; Lactate dehydrogenase, LDH; Creatine kinase, CK; *References of normal values (Merck Veterinary Manual 2010); **^{a,b} Different letters = significant differences between groups (P < 0.05).

but the value range in normal limits. Contrary to our results, Voicu *et al.* (2016) feeding fattening steers with two level of sorghum grains (15 % and, respectively 25 %) as substitute of barley, noticed no difference in the plasma triglycerides concentration while the level of plasma cholesterol significantly increased. Voicu *et al.* (2016) stated that this differences could be attributed to the structural particularity of the sorghum fat grains associated with the higher fat amount in the diet.

Plasma protein profile

The plasma protein profile provide valuable information on nutritional status and accurately reflect the protein consumption during a long period of time (Bhagavan and Chung-Eun Ha, 2015). From our knowledge little information's are available about the effects of dietary sorghum grain on plasma protein profile concentration of dairy cows (Baran *et al.* 2008; Bhagavan and Chung-Eun Ha, 2015; Nikkhah *et al.* 2004).

In our study, plasma protein profile of dairy cows fed with sorghum grain significantly increased (+10 %, P = 0.002) comparing to control diet, probably due to the increasing concentration of albumin fraction (+17 %, P = 0.013). However, the value for this two concentrations range in normal limits (Merck Veterinary Manual, 2010).

The other protein fractions (urea, creatinine and total bilirubin) were not affected (P > 0.05) by dietary treatment. In contrast to our results, Baran *et al.* (2008), by feeding 27 % grain sorghum in Holstein cattle did not found any significant differences in terms of serum total protein or albumin level. Nikkhah *et al.* (2004) observed an increased plasma urea concentration when fed cows with diets based on ground sorghum compared to cows fed other treatments.

Plasma mineral profile

Dietary replacement of barley grain with sorghum grain in dairy cows diet did not affect (P > 0.05) the plasma P and Fe concentrations. The calcium concentration increased (+21 %, P = 0.022), while the magnesium concentration decreased (-28 %, P = 0.010) in E diet compared to

C diet. Previous study of Emmanuel *et al.* (2007) have demonstrated that feeding cattle with diets rich in highly degradable carbohydrates results in a decreased plasma amount of Ca^{2+}, Fe^{2+}. Baran *et al.* (2008) stated that plasma mineral profile of beef cattle was not affected by feeding diets with 27 % sorghum grain inclusion.

Plasma enzymatic profile

In a normal physiological state, enzymes are involved in the process by which the body regulates its internal environment for chemical and biological processes. Thus, considerable variation in the enzyme parameters has been reported for bovine (Doornenbal *et al.*, 1988; Jenkins *et al.*, 1982; Peterson and Waldern, 1981) as effect of time, temperature or instability of the blood biochemical indicators.

The results of present study shown that the concentration of ALAT, ASAT, AP, GGT, LDH, and CK enzymes slightly increased as effect of fed sorghum grain, but the differences between treatments were not significant ($P > 0.05$). According to Bobe *et al.* (2004) the activities of enzymes are indicators of organ injury and can impact dairy cow's productivity. Similarly with our results, Voicu *et al.* (2016), reported that serum metabolites AP, GGT and CK were not affected by dietary inclusion of 15 % or 30 % sorghum grain in the fattening steer's diets. Nonetheless, plasma enzyme profile was within the normal physiological range for dairy cows (Merck Veterinary Manual, 2010).

CONCLUSION

Replacement of barley grain with sorghum grain in mid to late lactation dairy cow's diets had no adverse effect on animal's health state. Thus, 25 % of sorghum grain in the dairy cow's diet as alternative to energetically sources could be a good solution for farmers to feed the animals, especially in regions with limited irrigation water supplies.

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MILK PRODUCTION AND COMPOSITION OF LACTATING BUFFALOES FED RATIONS CONTAINING CORN SILAGE AND/OR FRESH BERSEEM

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ABSTRACT

Twelve lactating buffaloes in the 3rd to the 5th lactation, weighing 550-650 kg, after 8 weeks of calving in complete switch-back design with three treatments, were used in the study. Each group of buffaloes fed one of the three rations (R) consisted of 40 % concentrate feed mixture (CFM) and 20 % rice straw (RS) plus 40 % corn silage (CS, R1) or 40 % fresh berseem (FB, R2) or 20 % CS + 20 % FB (R3). Results showed that digestibility coefficients of dry matter (DM), organic matter (OM), crude protein (CP) and crude fibre (CF) as well as digestible crude protein (DCP) value were significantly ($P < 0.05$) higher, however, the digestibility coefficients of ether extract (EE) and nitrogen free extract (NFE), as well as total digestible nutrients (TDN) value were significantly ($P < 0.05$) lower for R2 compared to R1, while R3 was intermediate without significant differences. Actual and fat corrected milk (FCM; 7 %) yield were nearly similar for different experimental groups. However, buffaloes fed R1 had higher contents of milk fat and lactose, R2 had higher protein, solids-not-fat (SNF) and ash contents and R3 revealed higher total solid (TS) content ($P < 0.05$). The intake of DM was nearly similar for the different experimental groups. However, buffaloes fed R1 recorded significantly ($P < 0.05$) higher TDN intake and those fed R2 had higher DCP intake. Buffaloes fed R3 had the lower amount of DM, R2 and R3 had the lower amount of TDN and R1 had the lower amount of DCP required per one kg of 7 % FCM. Average daily feed cost and feed cost per one kg 7 % FCM were increased significantly ($P < 0.05$) with feeding fresh berseem compared to corn silage. Meantime, total revenue of milk yield was significantly ($P < 0.05$) higher with R3 compared to both R1 and R2. However, net revenue and economic efficiency increased significantly ($P < 0.05$) with corn silage compared to fresh berseem. The concentrations and excretion of macro and micro-elements in milk of buffaloes were significantly ($P < 0.05$) higher in R2 followed by R3, while R1 had the lower values. The concentrations of Ca, phosphorus (P), copper (Cu), Zn and Mn in milk of buffaloes fed corn silage were lower than the normal values.

Key words: lactating buffaloes; corn silage; fresh berseem; digestibility; milk yield and composition; feed conversion, milk minerals

INTRODUCTION

Using such high quality forage for feeding lactating buffaloes formulated balanced rations with adequate protein and energy that reflect on health conditions and enhanced milk production and composition (Mahmoud and Ebeid, 2014). Depending on corn silage as the main source of roughage in rations of lactating cows has an effect on animal productive performance similar to that in cattle depending on berseem hay plus corn silage in its ration (Orabi and Mousa, 2015).

Feeding management practices of the dairy farm can have a major impact on the levels of milk fat and protein concentration in milk. Nutritional strategies that optimize rumen function also maximize milk production and milk components. However, there are several strategies, which producers can use to enhance rumen function and the resulting milk components. However, nutritional

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strategies, which affect milk components, include adequate rumen degradable protein and adequate pounds of forage NDF in the diet especially for early lactation cows (Varga and Ishler, 2007).

Concentration of fat in milk can vary over a range of about 3 % through diet manipulation. In contrast, lactose, minerals, and the other solid contents of milk are not responsive, whereas the protein can vary at 0.6 % (Varga and Ishler, 2007).

Chemical composition of grass and legume are distinctively different. Crude protein (CP) content is generally lower for grass than legume; however, the composition of the crude protein differs. Grass contains more non-protein nitrogen in soluble protein and legumes contain more amino acids or peptides in soluble crude protein (Varga and Ishler, 2007).

A great deal of information has recently become available for better nutrition strategies including covering minerals to livestock, particularly lactating cows (McDowell and Valle, 2000). Minerals are greatly essential for proper metabolic functioning of the animal. A problem arises when the feed intake does not supply enough amount of minerals to meet the animal's requirements. This may occur when the feed is low in minerals, the bioavailability of the minerals is low, or another nutrient is interfering with the ability of the animal to absorb or utilize the minerals (Malmberg *et al*., 2003).

The contents of calcium, phosphorus, sodium, zinc and manganese were deficient in whole plant corn silage, and adding such minerals during feeding corn silage as a basal ration for feeding lactating cows is very necessary (Gaafar, 2009). Feeding of dairy cows with a ration containing 40 % concentrated feed mixture + 40 % corn silage + 20 % rice straw needs using mineral additives, especially for calcium, phosphorus, copper, zinc and manganese. The premix and seaweed additives increased apparent mineral absorption and retention, mineral concentration in hairs, blood plasma and milk (Bassiouni *et al*., 2013).

The objective of the present study was to investigate the effect of feeding rations containing corn silage and/or fresh berseem on feed intake, digestibility, milk yield and composition, mineral content in milk, feed conversion and economic efficiency of lactating buffaloes.

MATERIAL AND METHODS

The current work was carried out at private farm, Kafr El-Sheikh Province, North Delta, Egypt during the period from February to April 2018.

Experimental animals

Twelve lactating buffaloes at the 3rd to the 5th lactation, weighing 550-650 kg after 8 weeks of calving in complete switch-back design with three treatments, R1, R2 and R3 with three successive experimental periods were used. Each period consisted of 28 days and the first 14 days were considered as transition period followed by 14 days of the tested period, as described by Lucas (1956). Animals were divided into two blocks, each block contained 2 animals per each treatment as follows:

Three treatments (complete design):

	Block I	Block 2
Period 1	R1 R2 R3	R1 R2 R3
Period 2	R2 R3 R1	R3 R1 R2
Period 3	R1 R2 R3	R1 R2 R3

Lactating buffaloes were individually fed to cover the recommended requirements according to Kearl (1982). Rations were recalculated every week based on milk yield and body weight of animals.

Experimental rations and the management

Each group of buffaloes fed one of the three rations consisted of 40 % concentrate feed mixture (CFM) and 20 % rice straw (RS) plus 40 % corn silage (CS, R1) or 40 % fresh berseem (FB, R2) or 20 % CS + 20 % FB (R3). Concentrate feed mixture was given two times daily at 8 a.m. and 4 p.m., rice straw was offered two times at 9 a.m. and 5 p.m., while corn silage and fresh berseem were offered at 10 a.m. Buffaloes were watered three times daily at 7, 12 a.m. and 6 p.m. Chemical composition of feedstuffs and experimental rations are presented in Table (1).

Table 1. Chemical composition of feedstuffs and experimental rations

Item	Feedstuffs			Experimental rations			
	CFM**	CS	FB	RS	R1	R2	R3
Dry matter, %	90.45	27.72	16.3	89.96	65.26	60.69	62.98
Composition of DM, %							
Organic matter	91.20	92.84	87.45	83.62	90.34	88.18	89.26
Crude protein	16.46	8.14	15.87	2.78	10.40	13.49	11.94
Crude fiber	11.82	24.73	27.15	34.89	21.60	22.57	22.08
Ether extract	3.26	2.58	2.34	1.19	2.57	2.48	2.53
Nitrogen free extract	59.66	57.39	42.09	44.76	55.77	49.65	52.71
Ash	8.80	7.16	12.55	16.38	9.66	11.82	10.74

*CFM: concentrate feed mixture, CS: corn silage, FB: fresh berseem, RS: rice straw, R1: ration 1, R2: ration 2 and R3: ration 3.
**CFM was consisted of 35 % undecorticated cottonseed cake, 5 % linseed cake, 25 % ground yellow corn grains, 20 % wheat bran, 10 % rice bran, 3 % molasses, 1 % limestone and 1 % common salt.

Digestibility trials

Three digestibility trials were carried out during the 2nd period of feeding trial using the experimental buffaloes (four animals in each group) to determine nutrient digestibility coefficients and feeding values of the experimental rations using acid insoluble ash (AIA) as a natural marker (Van Keulen and Young, 1977). The samples of concentrate feed mixture, corn silage and rice straw were taken three times at the beginning, middle and the end of the collection period. Faeces samples were taken from the rectum of each cow twice daily at 12 h intervals during the collection period (7 days). Samples of feedstuffs and faeces were analysed according to A.O.A.C. (1995). The quantity of faeces was calculated from the equation given by Schneider and Flatt (1975) as follows:

Faeces DM (kg) = [DM intake (kg) x (100 – DM digestibility %)]/100

The urine samples were taken from each cow twice daily at 12 h intervals during the collection period (7 days) by clitoral stimulation after the vaginal area was washed with warm water and the urine volume was determined from the equation stated by Nennich *et al*. (2006) as follows:

Urine excretion ($kg.day^{-1}$) = (MUN × 0.563) + 17.1

Where, MUN was milk urea nitrogen and determined from the equation of Nousiainen *et al*. (2004) as follows:

MUN ($mg.l^{-1}$) = -14.2 + 0.17 × dietary CP content ($g.kg^{-1}$ dry matter).

Buffalo milking and milk samples

Individual morning and evening milk yield of lactating buffaloes were recorded daily and corrected for 7 % fat content (FCM) using the formula of 7 % FCM = 0.265 x milk yield (kg) + 10.5 x fat yield (kg) as stated by Raafat and Saleh (1962). Milk samples from consecutive evening and morning milking were taken at the 4th week of each period and mixed in proportion to yield. Milk fat, protein, lactose and total solids were determined using Milko-Scan (133B Foss Electric).

Preparation of samples for mineral determination

Wet ashing is primarily used in the preparation of milk samples for subsequent analysis of specific minerals according to (A.O.A.C., 1995). It breaks down and removes the organic matrix surrounding the mineral so that they are left in an aqueous solution. A quantity of 0.5 gram from the samples of feedstuffs and 1 ml from milk were wet ashing. Sample put in a flask with added 10 ml of pure sulfuric acid and then heated with added some drops of hydrogen peroxide. Heating is continued until the organic matter is completely digested, leaving only the mineral oxides. After that it was diluted to 100 ml by distilled water and kept in clean bottles for mineral determination. Mineral excretion in milk was calculated from the milk yield and the concentrations of minerals in milk of cows and buffaloes.

Mineral determination

- Calcium was determined according to the method of Baron and Bell (1957).
- Magnesium, copper, zinc, manganese and iron were determined by Atomic Absorption Spectrophotometer (G.B.C. Avanta).
- Phosphorus was determined by hydroquinone reagent using Spectrophotometer (Jenway 6305 UV/vis. Spectrophotometer).
- Sodium and potassium were determined by Flame Photometer (EEL).

Feed conversion

Feed conversion was calculated as the quantities of DM, TDN and DCP (kg) required to produce one kg 7 % FCM.

Economic efficiency

Economic efficiency of milk production was estimated and expressed as average daily feed cost, cost of 1 kg 7 % FCM, output of milk yield and the ratio between the output of milk yield and feed cost. The prices of one ton were 4500 LE for concentrate feed mixture, 600 LE for corn silage, 550 LE for fresh berseem and 425 LE for rice straw and one kg 7 % FCM was 10 LE according to the prices of 2018. (Ed. Note: 1 Egyptian Pound equals 0.053 Euro; exchange rate on June 4 2019.)

Statistical analysis

The data were analysed using general linear model procedure adapted by IBM SPSS Statistics (2014) for user's guide with one-way ANOVA. Significant differences in the mean values among dietary treatments were analysed by Duncan's test set at the level of significance $P < 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

Nutrient digestibility and feeding values

Digestibility coefficients and feeding values of the experimental rations are shown in Table (2). The digestibility coefficients of dry matter (DM), organic matter (OM), crude protein (CP) and crude fibre (CF) were significantly ($P < 0.05$) higher for R2 compared to R1, while R3 was intermediate without significant differences. However, the digestibility coefficients of ether extract (EE) and nitrogen free extract (NFE) were significantly ($P < 0.05$) higher for R1 compared to R2, while R3 was intermediate without significant differences.

In general the higher digestibility values obtained for most nutrients in all tested rations may be attributed to the effect of feeding such high quality forage (berseem or corn silage) which provided stimulatory factors to cellulolytic bacteria and other rumen bacteria. These factors resulted in some changes in the digestive function, which lead to increase in the availability and utilization of nutrients in the rumen and could have a significant impact on digestion and nutritive values of experimental rations.

Table 2. Nutrient digestibility coefficients and feeding values of experimental rations

Item	Experimental rations			MSE
	R1	R2	R3	
Digestibility coefficients, %				
DM	67.24^{b}	69.48^{a}	68.35^{ab}	0.56
OM	68.54^{b}	70.65^{a}	69.41^{ab}	0.55
CP	68.49^{b}	71.93^{a}	70.15^{ab}	0.69
CF	67.64^{b}	69.60^{a}	68.00^{ab}	0.55
EE	82.02^{a}	78.94^{b}	80.56^{ab}	0.70
NFE	72.50^{a}	68.54^{b}	70.62^{ab}	0.75
Feeding values, %				
TDN	66.91^{a}	63.85^{b}	65.20^{ab}	0.62
DCP	7.12^{b}	9.70^{a}	8.38^{ab}	0.39

$^{a, b}$ Values in the same row with different superscripts differ significantly ($P < 0.05$).

Data in the Table 2 show that R1 had significantly ($P < 0.05$) higher total digestible nutrient (TDN) value (66.91 %), followed by R3 (65.20 %), while R2 had significantly lower value (63.85 %). On the other hand, R2 had significantly ($P < 0.05$) higher digestible crude protein (DCP) value (9.70 %) followed by R3 (8.38 %), while R1 had significantly ($P < 0.05$) lower value (7.12 %). Higher TDN value of R1 may be due to the higher NFE content of corn silage than berseem. On the other hand, higher DCP value of R2 might be attributed to higher CP content of berseem than corn silage, as shown in Table 1. Generally, the present feeding values are mainly associated with the chemical composition and proportion of the experimental feedstuffs, in particular of berseem and corn silage. These results are in agreement with those obtained by El-Ready (2000), El-Aidy (2003) and Khalafalla *et al*. (2007), who found a higher digestibility of all nutrients for cows or buffaloe fed rations contained corn silage, berseem or corn silage and berseem along concentrate feed mixture.

Milk yield and composition

The effect of feeding tested rations on the actual and 7 % FCM yield of the experimental lactating buffaloes is shown in Table 3. The results showed no significant differences in both actual and 7 % FCM yield among the different experimental groups. Moreover, feeding experimental buffalo's rations contained either corn silage (R1) or fresh berseem (R2) or both tested forages (R3) did not affect these parameters. In addition, these results revealed that the requirements of buffaloes in the experimental groups were met by given formulated rations. Moreover, all experimental buffaloes, fed these tested rations, achieved and maintained higher milk production as a result of the feeding such high quality forage along with CFM in proper amounts and proportions. Similar results were found by El-Ready (2000), who reported that milk yield of dairy cows increased with feeding corn silage and fresh berseem. El-Aidy (2003) found that milk yield of buffaloes was not significantly affected by the partial replacement of berseem by corn silage.

In addition, there were significant ($P < 0.05$) differences in milk composition among animals fed tested rations. Feeding of corn silage in R1 significantly ($P < 0.05$) increased the content of milk fat and lactose compared to the feeding of fresh berseem in R2, while R3 showed the intermediate values with insignificant differences. Meantime, the buffaloes fed fresh berseem (R2) had significantly ($P < 0.05$) higher protein, solids-not-fat and ash contents compared with the feeding corn silage (R1) and R3 was intermediate without significant differences. On the other hand, R3 revealed significantly ($P < 0.05$) higher total solid content followed by R1, while R2 had the lower value. The high fat content of milk produced by the buffaloes fed R1 and R3 may be related to the inclusion of high energy corn silage in these two rations, while the lower content of milk produced by feeding fresh berseem in R2 may be attributed to the lower energy content. Milk fat depression can be alleviated within 7 to 21 days by changing the diet of the cow. Milk protein changes may take 3 to 6 weeks or longer if the problem has been going on for a long period (Grainger and Goddard, 2007). Balanced rations for lactating cows should contain at least 40 to 45 percent of ration dry matter from forage. This may be changed by addition of corn silage to the ration and the level of high fibre by-product feeds in the ration. Low forage intake can cause a major reduction in the fat content of milk due to low fibre levels (Mentin and Cook, 2006). Several potential reasons for low forage intake are inadequate forage feeding, poor quality forage and low neutral detergent fibre (NDF) content in forage, that was cut at a very immature stage or late in the fall stage (Bauman and Griinari, 2003). Target a forage NDF intake of 0.9 % of body weight daily. Although low forage diets increase milk protein production, this strategy is not recommended. The low forage levels contribute to acidosis and laminitis; it does not promote good health for the rumen of the cow in a long run. Protein and fat content also can be changed due to the physical form of forage being fed. Much of this is related to ration sorting and failure to provide a consistent diet throughout the day. Coarsely chopped silage and dry hay are the most common causes of sorting. At the extreme, very finely ground diets negatively affect rumen metabolism and depress fat and protein production. Monitoring ration particle size should be done to ensure that adequate effective fibre must be provided and total mixed rations (TMRs) must be mixed properly and distributed evenly to all cows (Dixon and Ernst, 2001). Forage quality can severely impact the amount of energy are being provided

Table 3. Milk yield and composition of buffaloes fed experimental rations

Item	Experimental rations			MSE
	R1	R2	R3	
Milk yield, kg.day^{-1}				
Actual milk	9.64	9.82	9.75	0.07
7 % FCM	9.60	9.39	9.56	0.11
Milk composition, %				
Fat	6.96^{a}	6.58^{b}	6.81^{ab}	0.07
Protein	3.42^{b}	3.75^{a}	3.64^{ab}	0.05
Lactose	5.25^{a}	5.04^{b}	5.16^{ab}	0.05
Solids not fat	9.75^{b}	10.03^{a}	9.97^{a}	0.08
Total solids	16.71^{ab}	16.61^{b}	16.78^{a}	0.11
Ash	1.08^{b}	1.24^{a}	1.17^{ab}	0.02

$^{a, b}$ Values in the same row with different superscripts differ significantly ($P < 0.05$).

in a ration. Therefore, in addition to doing forage test when new forages are harvested and fed consider having the laboratory to do digestibility measure of the forage as well. It can provide additional information that might shed light on whether lowered milk fat is due to highly fermentable carbohydrates in the ration or inadequate energy provided to the cows stemming from low forage quality. Improvement in nitrogen efficiency may affect milk components.

Feed intake

Average daily feed intake by lactating buffaloes is presented in Table 4. The intake of DM was nearly similar for the different experimental groups without significant difference. Although, the buffaloes fed R1 showed significantly ($P < 0.05$) higher TDN intake compared to those fed R2, the buffaloes fed R3 showed intermediate TDN with insignificant difference. DCP intake was significantly ($P < 0.05$) higher for R2 followed by R3, while R1 had significantly ($P < 0.05$) lower DCP intake. These results may be a reflection of the higher TDN content of R1 contained corn silage and higher DCP content of R2 contained fresh berseem (Table 2). Differences in cell wall fibre content (NDF) and fibre chemical structure are likely the reason for the superiority of legume silage. Legumes have a lower concentration of NDF but higher proportions of indigestible NDF and lignin than grasses. Due to lower NDF concentration and higher rate of rumen degradation rate of the digestible NDF in legumes than in grass, legumes have similar *in vitro* organic matter digestibility at much lower level of potentially digestible NDF than grasses (Weisbjerg *et al*., 2008). The intake of TDN by lactating buffaloes was significantly higher with feeding corn silage, however DCP intake was significantly higher with feeding fresh berseem in reflection to their chemical composition and their nutritive values (Mahmoud and Ebeid, 2014).

Feed conversion

Data of feed conversion expressed as DM, TDN and DCP required for producing one kg 7 % FCM are presented in Table 4. The amount of DM required for 1 kg of 7 % FCM was significantly ($P < 0.05$) lower for ration containing corn silage plus fresh berseem (R3) compared to the other two rations containing corn silage (R1) and fresh berseem (R2). The amount of TDN required for producing 1 kg 7 % FCM was significantly ($P < 0.05$) higher with feeding R1 compared with both R2 and R3. However, R2 showed significantly ($P < 0.05$) higher amount of DCP required for producing 1 kg of 7 % FCM followed by R3, while R1 had the lower value. The improvement of feed conversion ratio was reflected in the improvement in nutrient digestibility (Table 2), 7 % FCM yield (Table 3) and feed intake (Table 4). The better feed utilization was obtained at the combination of corn silage

Table 4. Feed intake, feed conversion and economic efficiency of buffaloes fed experimental rations

Item	Experimental rations			MSE
	R1	R2	R3	
Feed intake, kg/head/day				
DM	16.15	16.26	16.29	0.05
TDN	10.80[a]	10.38[b]	10.62[ab]	0.08
DCP	1.15[c]	1.58[a]	1.36[b]	0.06
Feed conversion, kg.kg^{-1} 7 % FCM				
DM	1.76[a]	1.76[a]	1.69[b]	0.02
TDN	1.17[a]	1.13[b]	1.10[b]	0.01
DCP	0.125[c]	0.171[a]	0.142[b]	0.007
Economic efficiency				
Feed cost, LE.day^{-1}	47.65[c]	55.84[a]	52.01[b]	1.21
Feed cost, LE.kg^{-1} 7 % FCM	5.18[c]	6.05[a]	5.40[b]	0.14
Total revenue, LE.day^{-1}	92.01[b]	92.24[b]	96.24[a]	0.97
Net revenue, LE.day^{-1}	44.36[a]	36.40[b]	44.23[a]	1.62
Economic efficiency	1.93[a]	1.65[b]	1.85[a]	0.05

[a, b, c] Values in the same row with different superscripts differ significantly ($P < 0.05$).

and fresh berseem. These results agreed with those obtained by El-Aidy (2003), who found that lactating buffaloes fed corn silage along with CFM were more efficient concerning the amount of 7 % FCM produced if compared to feeding berseem and CFM. El-Ready (2000) reported that feeding corn silage and fresh berseem improved the feed utilization efficiency in dairy cows. Dairy cows fed ration contained concentrate feed mixture, fresh berseem and corn silage showed the best feed conversion (Gaafar *et al*., 2010).

Economic efficiency

Results of economic efficiency shown in Table 4 revealed that both average daily feed cost and feed cost per 1 kg of 7 % FCM were significantly ($P < 0.05$) higher with feeding fresh berseem (R2) followed by feeding corn silage plus fresh berseem (R3), while feeding corn silage (R1) showed the lowest values. Meantime, total revenue of milk yield was significantly ($P < 0.05$) higher with R3 compared to both R1 and R2. Moreover, buffaloes fed both R1 and R3 had significantly ($P < 0.05$) higher net revenue and economic efficiency compared to those fed R2. These results agreed with those obtained by El-Aidy (2003), who found that lactating buffaloes fed corn silage along with CFM were more efficient economically compared to the feeding berseem and CFM. El-Ready (2000) reported that feeding corn silage and fresh berseem improved efficiency in dairy cows. Dairy cows fed ration contained concentrate feed mixture, fresh berseem and corn silage showed the best economic efficiency (Gaafar *et al*., 2010).

Mineral content of feedstuffs and experimental rations

Mineral contents of the experimental feedstuffs and experimental rations are presented in Table 5. The data revealed that the contents of Ca, K, Zn, Mn and Fe were higher in fresh berseem, while the contents of P, Mg, Na and Cu were higher in concentrate feed mixture. However, the lower contents of all mineral were detected in corn silage and rice straw. Fresh berseem, as legume, was rich in Ca content. Gaafar (2009) found that the contents of all minerals (Ca, P, Mg, Na, K, Cu, Zn, Mn and Fe) were higher in concentrate feed mixture compared to corn silage. Bassiouni *et al*. (2013) reported the low contents of calcium, phosphorus, magnesium, sodium, copper, zinc and manganese in corn silage and also the low contents of calcium, phosphorus, magnesium, potassium, copper and zinc in rice straw. Alfalfa has almost twice the ash

content of corn silage (NRC, 2001). Maize silage has low concentrations of calcium, magnesium, sodium and phosphorus. Feeding maize silage can exacerbate mineral deficiencies because magnesium and calcium already present in pasture diets. As a general rule, when maize silage makes up 25 % or more of a lactating cow diet, mineral supplementation is recommended. Depending on the individual farm, phosphorus supplementation may also be required. Requirements for trace minerals are similar when feeding maize silage or grazing pasture. A trace element supplementation or animal treatment programme should be routine 1 month before calving and 4 months after calving. Supplying the cow's requirements in copper, selenium, cobalt, iodine and zinc will cost approximately 4 cents per cow per day (Kolver *et al*., 2001).

The contents of all minerals were higher in R2 contained fresh berseem than in R1 contained corn silage, while mineral content in R3 was intermediate between R1 and R2. Mineral calculation of experimental rations showed that the contents of calcium, phosphorus, copper, zinc and manganese in R1 as well as phosphorus, copper and zinc in R3 were lower than the recommended requirements for dairy cows being 0.60, 0.40 %, 10, 40 and 40 ppm, respectively (NRC, 2001). Gaafar (2009) found that feeding growing calves with a ration containing corn silage needs mineral additives. Bassiouni *et al*. (2013) reported that feeding dairy cows with a ration containing 40 % concentrate feed mixture + 40 % corn silage + 20 % rice straw requires mineral additives, especially for calcium, phosphorus, copper, zinc and manganese.

Dietary mineral balance

Mineral balance in buffaloes fed experimental rations was presented in Table 6. The intake of macro- and micro- minerals was significantly higher ($P < 0.05$) with feeding R2 contained fresh berseem compared to R1 contained corn silage, whereas R3 was intermediate between them with significant differences. The higher increase was detected in Ca, medium increase was found in Na, K, Zn and Mn, low-medium increases in Mg and low increase in P and Cu. These increases might be attributed to the higher mineral content in fresh berseem compared to corn silage as well as in R2 compared to R1 (Table 5). The excretion in faeces and urine as well as the absorption and retention of all minerals increased significantly ($P < 0.05$) with increasing dietary mineral intake, which R2 showed significantly ($P < 0.05$) highest values followed by R3, whereas R1 showed lowest values. These results agreed with those obtained by Gaafar (2009), who found that dietary mineral intake, excretion, absorption and retention by growing Friesian calves decreased with increasing the level of corn silage in the rations. Bassiouni *et al*. (2013) reported that apparent absorption and retention of minerals by dairy cows increased with increasing mineral intake by seaweed and premix supplementation.

Table 5. Mineral contents of feedstuffs and experimental rations

Element	Feedstuffs			Experimental rations			
	CFM	CS	FB	RS	R1	R2	R3
Macro-element, %							
Calcium (Ca)	0.95	0.25	1.5	0.2	0.52	1.02	0.77
Phosphorus (P)	0.6	0.2	0.35	0.11	0.34	0.40	0.37
Magnesium (Mg)	0.45	0.13	0.35	0.11	0.25	0.34	0.39
Sodium (Na)	0.75	0.12	0.7	0.22	0.39	0.62	0.51
Potassium (K)	1.3	1.1	2.5	0.7	1.10	1.66	1.38
Micro-element, ppm							
Copper (Cu)	12	8	11	5	9.0	10.2	9.6
Zinc (Zn)	40	25	62	20	30.0	44.8	37.4
Manganese (Mn)	45	16	55	50	34.4	50.0	42.2
Iron (Fe)	450	245	470	340	346	436	391

Table 6. Dietary mineral balance in lactating buffaloes fed experimental rations

Element	Ration	Intake	Feces	Urine	Absorption	Retention
Macro-elements, g.day^{-1}						
Ca	R1	83.98^{c}	36.95^{c}	17.64^{c}	47.03^{c}	29.39^{c}
	R2	165.85^{a}	76.29^{a}	33.17^{a}	89.56^{a}	56.39^{a}
	R3	125.46^{b}	56.46^{b}	25.72^{b}	69.00^{b}	43.28^{b}
	MSE	12.07	5.81	2.29	6.26	3.98
P	R1	54.91^{c}	24.16^{c}	10.98^{b}	30.75^{c}	19.77^{c}
	R2	65.15^{a}	28.01^{a}	12.38^{a}	37.13^{a}	24.76^{a}
	R3	60.29^{b}	26.22^{b}	11.76^{ab}	34.06^{b}	22.31^{b}
	MSE	1.51	0.57	0.21	0.94	0.74
Mg	R1	40.08^{c}	18.04^{c}	8.02^{c}	22.04^{c}	14.03^{c}
	R2	55.39^{a}	25.48^{a}	11.63^{a}	29.91^{a}	18.28^{a}
	R3	48.88^{b}	22.24^{b}	10.02^{b}	26.64^{b}	16.62^{b}
	MSE	2.25	1.09	0.53	1.16	0.63
Na	R1	63.30^{c}	12.66^{c}	28.49^{c}	50.65^{c}	22.16^{c}
	R2	101.46^{a}	21.31^{a}	46.67^{a}	80.16^{a}	33.48^{a}
	R3	81.47^{b}	16.70^{b}	37.07^{b}	64.77^{b}	27.70^{b}
	MSE	5.66	1.28	2.70	4.37	1.68
K	R1	177.65^{c}	35.53^{c}	79.94^{c}	142.12^{c}	62.18^{c}
	R2	269.92^{a}	56.68^{a}	124.16^{a}	213.23^{a}	89.07^{a}
	R3	224.85^{b}	46.09^{b}	102.31^{b}	178.75^{b}	76.45^{b}
	MSE	13.60	3.12	6.52	10.48	3.96
Trace-elements, mg.day^{-1}						
Cu	R1	145.35^{c}	65.41^{c}	29.07^{c}	79.94^{c}	50.87^{b}
	R2	165.85^{a}	76.29^{a}	34.83^{a}	89.56^{a}	54.73^{a}
	R3	156.42^{b}	71.17^{b}	32.07^{b}	85.25^{b}	53.18^{a}
	MSE	3.04	1.61	0.85	1.43	0.58
Zn	R1	484.50^{c}	218.02^{c}	96.90^{c}	266.48^{c}	169.58^{c}
	R2	728.45^{a}	335.09^{a}	152.97^{a}	393.36^{a}	240.39^{a}
	R3	609.37^{b}	277.26^{b}	124.92^{b}	332.11^{b}	207.19^{b}
	MSE	35.96	17.27	8.28	18.69	10.42
Mn	R1	555.56^{c}	250.00^{c}	111.11^{c}	305.56^{c}	194.45^{c}
	R2	813.00^{a}	373.98^{a}	170.73^{a}	439.02^{a}	268.29^{a}
	R3	681.06^{b}	309.88^{b}	139.62^{b}	371.18^{b}	231.56^{b}
	MSE	38.10	18.36	8.84	19.74	10.91
Fe	R1	5587.90^{c}	2514.60^{c}	1117.60^{c}	3073.30^{c}	1955.80^{c}
	R2	7089.40^{a}	3261.10^{a}	1488.80^{a}	3828.30^{a}	2339.50^{a}
	R3	6370.70^{b}	2898.70^{b}	1306.00^{b}	3472.00^{b}	2166.00^{b}
	MSE	221.45	110.15	54.80	111.30	56.56

$^{a, b, c}$ Values in the column for each element with different superscripts differ significantly ($P < 0.05$).

Mineral concentrations in milk

The effect of feeding different rations on mineral concentrations in milk of buffaloes is shown in Table 7. The concentrations of macro-minerals (Ca, P, Mg, Na and K) and micro-minerals (Cu, Zn, Mn and Fe) in milk increased significantly ($P < 0.05$) with increasing dietary mineral intake, absorption and retention. Mineral concentrations

Table 7. Mineral concentrations in milk of buffaloes fed experimental rations

Element	Experimental rations			MSE
	R1	R2	R3	
Macro-mineral, g.kg^{-1}				
Ca	1.48^{b}	1.82^{a}	1.60^{ab}	0.08
P	1.00^{b}	1.30^{a}	1.10^{ab}	0.06
Mg	0.21^{b}	0.25^{a}	0.23^{ab}	0.01
Na	0.57^{b}	0.68^{a}	0.61^{ab}	0.03
K	1.58^{b}	1.82^{a}	1.66^{ab}	0.06
Micro-mineral, mg.kg^{-1}				
Cu	0.20^{b}	0.26^{a}	0.22^{ab}	0.02
Zn	3.64^{b}	4.16^{a}	3.82^{ab}	0.12
Mn	0.042^{b}	0.053^{a}	0.046^{ab}	0.003
Fe	1.27^{b}	1.43^{a}	1.33^{ab}	0.04

$^{a, b}$ Values in the same row with different superscripts differ significantly (P < 0.05).

in milk were significantly higher (P < 0.05) in R2 than in R1, while R3 was intermediate without significant differences. The high significant positive correlations were observed between dietary mineral retention and their concentrations in milk were following: Ca = 0.90, P = 0.85, Mg = 0.80, Na = 0.88, K = 0.85, Cu = 0.86, Zn = 0.89, Mn = 0.85 and Fe = 0.80. The concentrations of Ca and P in milk of buffaloes fed R1 and R3 were lower than the normal values being 1.63 and 1.11 g.kg^{-1}, respectively (Soliman, 2005). Bassiouni *et al*. (2013) found that macro and micro-mineral concentration in the milk of cows fed rations containing 40 % corn silage increased with 25 g premix or 50 g seaweed (as a source of minerals) per head per day.

Table 8. Mineral excretion in milk of buffaloes fed experimental rations

Element	Experimental rations			MSE
	R1	R2	R3	
Macro-mineral, g.kg^{-1}				
Ca	13.68^{b}	17.56^{a}	15.71^{ab}	0.81
P	9.24^{b}	12.55^{a}	10.80^{ab}	0.62
Mg	1.94^{b}	2.41^{a}	2.23^{ab}	0.11
Na	5.27^{b}	6.56^{a}	5.96^{ab}	0.27
K	14.60^{b}	17.56^{a}	16.27^{ab}	0.61
Micro-mineral, mg.kg^{-1}				
Cu	1.85^{b}	2.51^{a}	2.19^{ab}	0.16
Zn	33.63^{b}	40.14^{a}	37.51^{ab}	1.31
Mn	0.39^{b}	0.51^{a}	0.45^{ab}	0.03
Fe	11.73^{b}	13.80^{a}	13.03^{ab}	0.45

$^{a, b}$ Values in the same row with different superscripts differ significantly (P < 0.05).

Mineral excretion in milk

The excretion of macro and micro-minerals in milk of buffaloes fed experimental rations is presented in Table 8. The amounts of all mineral excretion in milk were significantly higher ($P < 0.05$) with feeding R2 compared to R1, whereas R3 was intermediate without significant differences. These results illustrate that mineral excretion in milk increased with increasing dietary mineral intake, absorption and retention. Average value and range of mineral excretion in milk as a percentage of mineral retention in the body were high for P - 48.84 % (26.92-69.90 %) and Ca - 36.91% (20.04-51.78 %); medium for Na - 21.35 % (10.67-36.85 %), K - 21.24 % (11.13-35.81 %), Zn - 18.01 % (12.48-27.18 %) and Mg - 13.42 % (7.94-19.26 %); low for Cu - 4.17 % (1.47-7.82 %) and very low for Fe - 0.60 % (0.31-0.89 %) and Mn - 0.19 % (0.10-0.36 %). These results revealed that milk is a good source of calcium, phosphorus, sodium, potassium, zinc and magnesium. Many minerals in milk are associated together in the form of salts, such as calcium phosphate. In milk, approximately 67 % of calcium, 35 % of magnesium, and 44 % of phosphorus are salts bound within the casein micelle and the remainder are soluble in the serum phase. The fact that calcium and phosphorus are associated as salts bound with the protein does not affect the nutritional availability of either calcium or phosphate. Milk contains small amounts of copper, iron, manganese and sodium and is not considered a major source of these minerals in the diet. The concentration of major elements depends on the animal species, method of feeding, lactation stage and health state of the udder (Cashman, 2006).

CONCLUSION

The present results indicated that the use of high quality forage, such as corn silage and fresh berseem for feeding lactating buffaloes formulated balanced rations with adequate protein and energy that reflect enhanced digestibility, feed intake, milk yield and composition, feed conversion and economic efficiency, mineral balance and minerals excretion in milk compared to feeding corn silage or fresh berseem alone.

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FATTY ACIDS IN THE INTRAMUSCULAR FAT OF BERRICHON DU CHER AND SUFFOLK HEAVY LAMBS KEPT IN SEMI-INTENSIVE PRODUCTION SYSTEMS IN SLOVAKIA

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ABSTRACT

The objective of this study was to determine the content of fatty acids (FAs) in the intramuscular fat of heavy lambs of two breeds: Berrichon du Cher (BE) and Suffolk (SF) in a semi-intensive production system with different nutrition management schemes applied (SI1 and SI2) using gas chromatography. Nutrition differed mainly in a short period before the slaughter: BE/SI1 lambs were fed with hay and concentrates, SF/SI2 lambs grazed and suckled a milk. The samples were taken from the *Musculus longissimus dorsi* and the analysis of variance with factors of breed/production system (BE/SI1, SF/SI2) and lamb sex (males, females) was used to study the differences in FAs. The content of essential FAs, linoleic acid and α-linolenic acid summed, was higher in SF/SI2 lambs (6.26 g.100 g^{-1} FAME); this significantly ($P < 0.001$) differed from BE/SI1 lambs (4.64 g.100 g^{-1} FAME). The contents of health beneficial FAs (arachidonic, eicosapentaeonic, docosapentaeonic, docosahexaenoic acids) were also higher in SF/SI2 lambs (2.00, 0.59, 0.83, 0.27 g.100 g^{-1} FAME) and significantly ($P < 0.001$) differed from BE/SI1 lambs (1.15, 0.30, 0.44, 0.13 g.100 g^{-1} FAME). The content of conjugated linoleic acid (health beneficial FA as well) was 1.67 g.100 g^{-1} FAME in SF/SI2 lambs and 1.07 g.100 g^{-1} FAME in BE/SI1lambs ($P < 0.001$). The ratio of *n-6/n-3* polyunsaturated FAs agreed, whilst the ratio of polyunsaturated/saturated FAs did not agree with the recommended values (found better in SF/SI2 lambs).

Key words: sheep; production system; lamb sex; *Musculus longissimus dorsi*; fatty acids

INTRODUCTION

Worldwide, research is focused on revealing the potential benefits from the consumption of lamb meat (Swanson *et al.*, 2012, Mortimer *et al.*, 2014, Ponnampalam *et al.*, 2014). There are many studies assessing the quality of lamb meat on the base of essential fatty acids (FAs), e.g. linoleic acid, α-linolenic acid and health promoting polyunsaturated FAs (PUFA) in the intramuscular and subcutaneous fat (Mortimer *et al.* 2014, Ponnampalam *et al.* 2014). Regarding PUFA, eicosapentaenoic acid and docosahexaenoic acid are believed to be of anti-inflammatory effect, helping to protect the human body against autoimmune diseases (Simopoulos, 2002, McAfee *et al.*, 2010) and to be linked to healthy aging throughout life (Swanson *et al.*, 2012). Conjugated linoleic acid was investigated as well for its anti-carcinogenic, anti-atherosclerotic and

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anti-diabetic effects (Raes *et al.*, 2004; Serra *et al.* 2009). Regarding saturated FAs (SFA), high amounts of myristic acid and palmitic acid are assumed to increase the risk of cardiovascular diseases and of the higher cholesterol level (Howes *et al.*, 2015). On the contrary, high amounts of stearic acid are considered to have no an effect on cholesterol level (Howes *et al.*, 2015). The research was also focused on the determination of recommended values for ratios of PUFA/SFA to be beneficiary for human health: above 0.7 or 0.45 – according to Raes *et al.* (2004) and Williams (2000), or a minimum of 0.4 – according to Wood and Enser (1997), as well as *n-6*/*n-3* PUFA, below 4 – according to Simopoulos (2002) and Wood *et al.* (2003). Investigation of essential FAs revealed that their health beneficial effect depends on both production system and nutrition of lambs (Fisher *et al.*, 2000, Sinanoglou *et al.*, 2013). Díaz *et al.* (2005), Aurousseau *et al.* (2007) and Nuernberg *et al.* (2008) also reported that the content of FAs depends on quality of pasture, hay/silage and concentrates. The content of FAs may also be affected by a genotype of animals; this is believed to be of lesser influence (Santos-Silva *et al.*, 2002, Ponnampalam *et al.*, 2014). Sanudo *et al.* (2000) reported that effects attributed to breed are often due to the degree of fatness, live weight, slaughter age or the production system.

In Slovakia, breeding of various meat and/or non-dairy dual-purpose breeds producing heavy lambs of carcass weight above 13 kg is increasing being about 10 to 15 % of total number of sheep (according to Margetín *et al.*, 2018). Three production systems for fattening of heavy lamb are applied in Slovakia. Traditionally, the indoor lambing system is applied in winter. Lambs are fed with complex feed rations, including hay/silage and concentrate and housed in stables. Since 1990s, a pasture production system becomes important. It is characterised by indoor lambing in spring; lambs are moved with ewes to pasture at an early age, fed with no hay/silage and concentrates. Regardless of a system, lambs are allowed to suckle milk. In addition to these two systems, a semi-intensive production system, as a combination of traditional and pasture systems with indoor lambing, is applied. Lambs with ewes are housed at an early age; nutrition of ewes consists of hay and concentrates; lambs are allowed to suckle milk. When pasture is available, lambs and ewes are pastured; lambs are offered with concentrates when needed. Two weeks before slaughter, lambs are either fed with hay/concentrate or lambs suckle milk and graze. Some research, focused on the analyses of the content of FAs in the intramuscular fat of light and heavy lambs, was done in Slovakia (Margetín *et al.* 2014; 2018). These studies were limited to pastured and stabled animals and did not cover the semi-intensive production system.

Therefore, the objective of this study was to determine the content of FAs in the intramuscular fat (analysed from *Musculus longissimus dorsi* samples) of heavy lambs of two breeds: Berrichon du Cher (BE) and Suffolk (SF) in the semi-intensive production system with different nutrition management schemes applied (SI1 and SI2). In addition to the influence of the overlapping breed/production system factor (BE/SI1 and SF/SI2), the influence of the lamb sex factor on the content of FAs was also investigated.

MATERIAL AND METHODS

Animals and production system

Two groups of heavy lambs (each included 20 heads: 13 males and 7 females): Berichon du Cher (BE) and Suffolk (SF) from two flocks – the semi-intensive production system differing in nutrition management (SI1 and SI2) – were included in the experiment. This design was the only available due to the fact that commercial flocks with an identical semi-intensive system for different breeds cannot be found in Slovakia. The distance between flocks was about 15 km (GPS coordinates of location 1 were 48°43' N and 19°96' E; GPS coordinates of location 2 were 48°34' N and 20°06' E). Both flocks were characterised by a similar height above the sea, annual rainfall and average temperature. The pasture was natural, free of any seed enrichment. The fence system (ewes and lambs grazed together) was applied. Flock size was 94 and 203 breeding females, respectively.

The first group consisted of BE lambs. Ewes lambed indoors, mainly in April; their diet consisted of 2 kg of hay (mixture of alfalfa and grass hay), 3 kg of alfalfa silage and 200 g of oat per head per day. Since birth, lambs were housed with ewes in stable

(maternity pens and nurseries, respectively) and suckled milk. From two to three weeks after birth, in addition to milk, lambs were fed with on-farm grained oat and barley (ratio 1:1) per head per day. Since three weeks after parturition, lambs and ewes were moved to pasture. Lambs were offered on-farm grained oat and barley (100 to 200 g per head per day) when needed. Two weeks before slaughter, lambs were separated from ewes and allowed neither to graze nor suckle milk. They were fed with 200 g of grained oat and barley (ratio 1:1) per head per day; hay was available *ad libitum*. This breed/production system is referred to as BE/SI1 lambs.

The second group consisted of SF lambs. Ewes were lambed indoors, mainly in April; their diet included 2 kg of hay (mixture of alfalfa and grass hay), 3 kg of alfalfa silage and 400 g of concentrates per head per day. Since birth, lambs were housed with ewes in stable (maternity pens and nurseries, respectively) and suckled milk. Until three weeks of age, lambs were also fed with a commercial starter PURINA (Agribrands Europe, Hungary) *ad libitum*, which consisted of dry matter (88 %), NL (16 %), fat (2.2 %), fibre (11 %), Ca (1.3 %), P (0.4 %) and Na (0.3 %) and supplements. Since three weeks after parturition, ewes and lambs were moved to pasture. Instead of PURINA, lambs were offered on-farm grained oat and barley (100 to 200 g per head per day) when needed. Two weeks before slaughter, lambs were only allowed to graze and suckle milk. This breed/production system is referred to as SF/SI2 lambs.

Lambs of both groups were slaughtered in the authorised slaughterhouse run by the National Agricultural and Food Centre – Research Institute of Animal Production Nitra. The average weight before slaughter was 31.8 ± 3.4 kg (BE/S1) and 36.1 ± 5.0 kg (SF/SI2), respectively. The average age of lambs was 86 ± 2.9 days (BE/S1) and 93 ± 6.8 days (SF/SI2), respectively. The average daily gain of lambs was 290 ± 40 g (BE/S1) and 330 ± 50 g (SF/SI2), respectively.

Analysis of fatty acids

Twenty-four hours after slaughter, meat samples were taken from the *Musculus longissimus dorsi* (MLD) between the 9th and 13th vertebra. The analysis of the content of fatty acids (FAs) in the intramuscular fat (IMF) was undertaken in the laboratory of the Institute of Chemistry (Faculty of Natural Sciences at Comenius University in Bratislava), following the procedure described in the study of Margetín *et al.* (2018).

A total of 70 FA were identified. The hypocholesterolaemic FA/hypercholesterolaemic FA ratio (h/H ratio) was calculated according to Santos-Silva *et al.* (2002) and Sinanoglou *et al.* (2013). The atherogenic index (AI) and thrombogenic index (TI) were calculated according to Ulbricht and Southgate (1991) and Sinanoglou *et al.* (2013).

Statistical analysis

Data were analysed using an analysis of variance. General Linear Model procedure as implemented in programme SAS (2009) was applied. The model included: (A) the overlapping breed/production system factor (BE/SI1 and SF/SI2 lambs) due to the fact that variance of breed was hardly possible to be distinguished from variance of production system, and (B) the lamb sex factor (males and females). A preliminary analysis, which included breed/production system–sex interaction, revealed its non-significant influence; thus, this was not considered. Differences in estimated least square means of individual levels of factors included were tested using a Scheffe test and were considered statistically significant in case of $P < 0.05$ or $P < 0.001$.

RESULTS AND DISCUSSION

Analysis of individual fatty acids

Effect of breed/production system

The contents of FAs in IMF of MLD samples from lambs of analysed breed/production systems are shown in Table 1. Regarding individual saturated FAs (SFA), only palmitic acid (PA) was found to be significantly different ($P < 0.001$) between BE/SI1 lambs (23.40 g.100 g^{-1} FAME) and SF/SI2 lambs (21.92 g.100 g^{-1} FAME). In accordance with the studies of Díaz *et al.* (2005), Fiori *et al.* (2013) and Cividini *et al.* (2014), PA was the most common SFA. Although, it is impossible to distinguish between variance accounted for breed and variance accounted for production system, both values were

found within the range as estimated for pastured (21.80 g.100 g^{-1} FAME) and stabled (28.51 g.100 g^{-1} FAME) Ile de France (IF) lambs by Margetín *et al.* (2018). This probably reflects the fact that both breeds in the semi-intensive production systems were allowed to graze and/or had less concentrate supplements in their diet. Moreover, it seems that the content of PA in SF/SI2 lambs decreased to greater extent due to the fact that these lambs were pastured also in the last period of fattening. The content of PA in this study was found to be similar to the content of PA in commercial crossbred castrated males from the United Kingdom (breeds used for this crossbreeding were not given) and male crossbreds between Sufolk or Schwarzköpfe and Merino Landschaf from Germany (both on grass and concentrate supplements), when compared with the findings of Díaz *et al.* (2005). No differences ($P > 0.05$) were found when the contents of remaining SFA i.e. lauric acid, myristic acid, margaric acid and stearic acid (SA) were compared. The contents of these were found to be similar to the values in pastured IF lambs (Margetín *et al.*, 2018). In accordance with the studies of Díaz *et al.* (2005), Fiori *et al.* (2013) and Cividini *et al.* (2014), SA was the second common SFA. In SF/SI2 lambs, this was almost same as in stabled IF lambs (14.46 vs. 14.51 g.100 g^{-1} FAME). In BE/SI1 lambs, this was almost the same as in pastured IF lambs (15.28 vs. 15.65 g.100 g^{-1} FAME), when compared with the study of Margetín *et al.* (2018).

Regarding individual mono-unsaturated FAs (MUFA), oleic acid (OA), *trans*-vaccenic acid (TVA) and palmitoleic acid were found to be of significantly different ($P < 0.001$) content between BE/SI1 and SF/SI2 lambs. The most common MUFA was found OA; its lower content was found in SF/SI2 lambs (31.83 g.100 g^{-1} FAME) than in BE/SI1 lambs (34.02 g.100 g^{-1} FAME). The values reported in this study were slightly higher than Aurousseau *et al.* (2007) reported for lambs on pasture, those diets were enriched with concentrates for a short/long period

Table 1. Least square means of fatty acids in the intramuscular fat (g.100g^{-1} fatty acid methyl esters) of lamb meat

Fatty acids	Breed/Production system		Sex		SEM	R^2
	BE/SI1	SF/SI2	Male	Female		
C12:0 (lauric)	0.59	0.51	0.56	0.55	0.188	0.08
C14:0 (myristic)	5.26	4.80	4.79	5.27	1.111	0.11
C16:0 (palmitic)	23.40^{a}	21.92^{b}	22.25	23.07	1.573	0.26
C17:0 (margaric)	0.98	0.95	0.95	0.98	0.076	0.08
C18:0 (stearic)	15.28	14.46	15.70^{A}	14.04^{B}	1.677	0.25
C16:1 *cis 9* (palmitoleic)	0.52^{a}	0.57^{b}	0.53	0.56	0.057	0.20
C 18:1 *trans 9* (elaidic)	0.26	0.27	0.26	0.27	0.025	0.03
C18:1 *cis 9* (oleic)	34.02^{a}	31.83^{b}	32.48	33.37	2.185	0.28
C18:1 *trans 11* (TVA)	2.11^{A}	2.93^{B}	2.46	2.58	0.308	0.70
C18:2 *n-6* (linoleic)	3.85^{a}	5.08^{b}	4.77	4.16	1.428	0.24
C18:3 *n-6* (GLA)	0.04	0.04	0.05	0.04	0.019	0.08
C18:3 *n-3* (ALA)	0.78^{A}	1.19^{B}	1.01	0.96	0.212	0.56
C18:2 *cis9 trans 11* (RA)	0.96^{A}	1.51^{B}	1.15^{a}	1.32^{b}	0.256	0.57
C20:4 *n-6* (arachidonic)	1.15^{a}	2.00^{b}	1.80	1.36	0.975	0.23
C20:5 *n-3* (EPA)	0.30^{A}	0.59^{B}	0.52	0.37	0.255	0.36
C22:5 *n-3* (DPA)	0.44^{A}	0.83^{B}	0.72	0.55	0.313	0.39
C22:6 *n-3* (DHA)	0.13^{A}	0.27^{B}	0.22	0.18	0.110	0.37

BE/SI1: Berrichon du Cher in semi-intensive system 1; SF/SI2: Suffolk in semi-intensive system 2; SEM: Standard error of mean; R^2: Coefficient of determination.
TVA: *trans*-vaccenic acid; ALA: α-linolenic acid; GLA: γ-linolenic acid; RA: rumenic acid; EPA: eicosapentaeonic acid; DPA: docosapentaeonic acid; DHA: docosahexaeonic acid.
$^{A, B:}$ differences between individual levels of factors at $P < 0.001$; $^{a, b:}$ differences between individual levels of factors at $P < 0.05$.

(28.8 and 29.9 g.100 g^{-1} FAME). Contrariwise, they were slightly lower, than Silva Sobrinho *et al.* (2014) reported for meat of lambs on a diet with a forage/concentrate ratio (1:1). The values reported in this study were higher than Margetín *et al.* (2018) reported for both pastured and stabled lambs. According to Aurousseau *et al.* (2007), it may be expected that the more concentrate in a diet, the more absorption of OA is found. Jenkins (1994) also reported that OA in meat of stabled lambs fed with higher amount of concentrates should be of higher content than in meat of grazed lambs fed with lower amount of concentrates. Nevertheless, OA is mobilised from body fat and its higher contents in both BE/SI1 and SF/SI2 lambs are probably due to the fact that daily gains of these breeds were found higher than daily gains of stabled IF lambs (Margetín *et al.*, 2018). The content of TVA was 2.11 g.100 g^{-1} FAME (BE/SI1 lambs) and 2.93 g.100 g^{-1} FAME (SF/SI2 lambs), respectively. These values were lower than those found in pastured IF lambs, but higher than those found in stabled IF lambs (Margetín *et al.*, 2018). Moreover, the findings about higher contents of TVA (most important precursor of conjugated linoleic acid (CLA)) in meat of grazed lambs (regardless of access to concentrates) agree with the studies of Nuernberg *et al.* (2005) and Aurousseau *et al.* (2007), who found higher contents of TVA in grazed lambs as well. The content of palmitooleic acid was 0.52 g.100 g^{-1} FAME (BE/SI1 lambs) and 0.57 g.100 g^{-1} FAME (SF/SI2 lambs), respectively. Elaidic acid, contrariwise to remaining individual MUFA, showed no difference ($P > 0.05$) between meat of two lamb groups (0.26 and 0.27 g.100 g^{-1} FAME). Similarly, Margetín *et al.* (2018) reported no difference between meats of pastured and stabled IF lambs (0.28 g.100 g^{-1} FAME, both) as far as the content of this individual MUFA is related.

Regarding individual polyunsaturated FAs (PUFA), γ-linolenic acid (GLA) was PUFA of the lowest content in analysed lamb groups (0.04 g.100 g^{-1} FAME in both, i.e. no difference observed, $P > 0.05$). The remaining individual PUFA significantly differed ($P < 0.001$, $P < 0.05$) between these two lamb groups. The essential linoleic acid (LA) was PUFA of the highest content i.e. 3.85 g.100 g^{-1} FAME (BE/SI1 lambs) and 5.08 g.100 g^{-1} FAME (SF/SI2 lambs), respectively. The content of essential α-linolenic acid (ALA) was 0.78 g.100 g^{-1} FAME (BE/SI1 lambs) and 1.19 g.100 g^{-1} FAME (SF/SI2 lambs), respectively. The content of rumen acid (RA) was 0.96 g.100 g^{-1} FAME (BE/SI1 lambs) and 1.51 g.100 g^{-1} FAME (SF/SI2 lambs). The contents of health beneficial PUFA i.e. arachidonic acid (AA), eicosapentaeonic acid (EPA), docosapentaeonic (DPA) and docosahexaeonic acid (DHA) were following: 1.15, 0.30, 0.44 and 0.13 g.100 g^{-1} FAME (BE/SI1 lambs) and 2.00, 0.59, 0.83 and 0.27 (SF/SI2 lambs). Except for ALA and AA, the remaining PUFA followed the expected pattern and fell within the range given by values for stabled and pastured IF animals (Margetín *et al.*, 2018). In general (with few exceptions), meat of lambs partly allowed to graze (regardless of breed) is of more favourable content of FAs (individual SFA, MUFA and PUFA investigated) than the meat of stabled lambs, although this is of slightly lower quality than the meat of lambs grazed, with no concentrates in their diet).

Effect of lamb sex

The content of FAs, as affected by lamb sex, is shown in Table 1. Comparisons within individual SFA, MUFA and PUFA revealed significant differences ($P < 0.001$, $P < 0.05$) between males and females only in the contents of SA and RA, i.e. SA was found higher in males (15.70 g.100 g^{-1} FAME) than in females (14.04 g.100 g^{-1} FAME), while RA was found higher in females (1.32 g.100 g^{-1} FAME) than in males (1.15 g.100 g^{-1} FAME), following the same tendency as observed by Margetín *et al.* (2018). Regarding remaining individual SFA, MUFA and PUFA, no pattern in their content was found. About half of FAs tended to be of higher content in males, but these difference were negligible.

Analysis of groups of fatty acids, their ratios and indexes

Effect of breed/production system

The contents of FA groups (SFA, MUFA, PUFA, etc.), their ratios and indexes (as affected by breed/production system) are shown in Table 2. The content of SFA group was 48.59 g.100 g^{-1} FAME (BE/SI1 lambs) and 45.57 g.100 g^{-1} FAME (SF/SI2 lambs), respectively. The content of MUFA group was 42.10 g.100 g^{-1} FAME (BE/SI1lambs) and 40.77 g.100 g^{-1} FAME (SF/SI2 lambs), respectively. The content of PUFA group was 9.30 g.100 g^{-1} FAME (BE/SI1 lambs) and 13.66 g.100 g^{-1} FAME (SF/SI2 lambs), respectively. Except for MUFA group, the differences were significant ($P < 0.001$).

Except for SFA group (both values similar to pastured IF lambs), the values for MUFA and PUFA groups were within the values for stabled and pastured IF animals as reported by Margetín *et al.* (2018). The values of SFA, MUFA and PUFA groups were also similar to the values reported by Díaz *et al.* (2005) for male crossbreds (grazed and fed with concentrates) from the United Kingdom and Germany. Health benefits of lamb meat (similarly to other types of red meat), were questioned due to its relatively high content of SFA and relatively low content of PUFA (McAfee *et al.*, 2010, Howes *et al.*, 2015). Regarding contents of *cis*-UFA (38.06 and 36.59 g.100 g^{-1} FAME in BE/SI1 and SF/SI2 lambs) and *trans*-UFA (4.84 and 5.65 g.100 g^{-1} FAME in BE/SI1 and SF/SI2 lambs), these were similar to values for stabled (on a diet with concentrates) and pastured IF lambs (for comparison, see Margetín *et al.*, 2018). The contents of branched-chain FAs were 1.95 g.100 g^{-1} FAME (BE/SI1 lambs) and 1.80 g.100 g^{-1} FAME (SF/SI2 lambs) and were higher than Aurousseau *et al.* (2007) reported for grazed IF male crossbreds, fed with hay and concentrates either a short (22 days) or long (41 days) period before

Table 2. Least square means of fatty acid groups, their ratios and indexes in the intramuscular fat (g.100g^{-1} fatty acid methyl esters)

Fatty acids	Breed/Production system		Sex		SEM	R^2
	BE/SI1	SF/SI2	Male	Female		
SFA[1]	48.59^{A}	45.57^{B}	47.23	46.94	2.630	0.31
MUFA[2]	42.10	40.77	40.64	42.22	2.457	0.17
PUFA[3]	9.30^{A}	13.66^{B}	12.13	10.83	3.248	0.39
Trans-UFA[4]	4.84^{A}	5.65^{B}	5.10^{a}	5.38^{b}	0.424	0.55
Cis-UFA[5]	38.06	36.59	36.60	38.05	2.386	0.19
BCFA (*iso, anteiso*)[6]	1.95^{a}	1.80^{b}	1.82	1.94	0.195	0.21
Essential FA (LA+ALA)	4.64^{A}	6.26^{B}	5.79	5.11	1.592	0.30
n-6 PUFA[7]	5.18^{a}	7.33^{b}	6.81	5.70	2.486	0.24
n-3 PUFA[8]	1.76^{A}	3.01^{B}	2.62	2.16	0.883	0.44
CLA[9]	1.07^{A}	1.67^{B}	1.28^{a}	1.46^{b}	0.274	0.58
PUFA/SFA	0.19^{A}	0.30^{B}	0.26	0.23	0.087	0.37
∑*n-6* /∑*n-3* PUFA	2.94^{A}	2.40^{B}	2.72	2.62	0.301	0.50
LA/ALA	4.98^{a}	4.22^{b}	4.84	4.36	0.875	0.23
LC *n-6* /LC *n-3* PUFA[10]	1.32^{a}	1.18^{b}	1.29	1.22	0.189	0.18
AI (atherogenic index)	0.93	0.82	0.85	0.89	0.159	0.16
TI (thrombogenic index)	1.51^{A}	1.25^{B}	1.37	1.39	0.184	0.41
h/H[11] index	1.44	1.58	1.56	1.46	0.240	0.15

[1]SFA is the sum of saturated fatty acids: C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + *iso*C14:0 + C14:0 + *iso*C15:0 + *anteiso*C15:0 + C15:0 + *iso*C16:0 + C16:0 + *iso*C17:0 + *anteiso*C17:0 + C17:0 + *iso*C18:0 + C18:0 + C19:0 + C20:0 + C21:0 + C22:0; [2]MUFA, sum of monounsaturated FA: C12:1 + C14:1 + *t*C16:1 + *c*C16:1 + 9*c*C16:1 + C17:1 + 6-8*t*C18:1 + 9*t*C18:1 + 10*t*C18:1 + 11*t*C18:1 + 12*t*C18:1 + 9*c*C18:1 + (15*t*+11*c*C18:1) + 12*c*C18:1 + 13*c*C18:1 + (14*c*C18:1+9*t*12*t*18:2 / 2) + 15*c*C18:1 + (C18:2+C19:1 / 2) + C20:1; [3]PUFA is the sum of polyunsaturated FA: (14*c*C18:1+ 9*t*12*t*18:2 / 2) + 9*c*13*t*C18:2 + (8*t*13*c*+9*c*12*t*C18:2) + (9*t*12*c* + 11*t*15*c*C18:2) + C18:2*n-6* + 9*c*15*c*C18:2 + 12*c*15*c*C18:2 + *cc*C18:2 + *cc*C18:2 + (C18:3 *n-6* GLA) + (C18:2+C19:1 / 2) + cyklo + 9*t*12*c*15*c*C18:3 + C18:3 *n-3* + (9*c*11*t*C18:2 CLA) + *ct*CLA + *cc*CLA + *tc*CLA + *tt*CLA + C18:3 + C20:2 + C20:3 *n-9* + C20:3 *n-6* + C20:4 *n-6* + C20:3 *n-3* + C20:4 *n-3* + C20:5 *n-3* + furyl C22 + C22:4 *n-3* + C22:5 *n-3* + C22:6 *n-3*; [4]*Trans* UFA is the sum of *trans*UFA: *t*C16:1 + 6-8*t*C18:1+ 9*t*C18:1+ 10*t*C18:1 + 11*t*C18:1 + 12*t*C18:1 +((15*t*+11*c*C18:1/3)*2) + (14*c*C18:1+ 9*t*12*t*18:2 / 2) + 9*c*13*t*C18:2 + (8*t*13*c*+9*c*12*t*C18:2) + 9*t*12*c*+11*t*15*c*C18:2 + *tt*CLA; [5]Cis-UFA is the sum of cis-UFA: *c*C16:1 + 9*c*C16:1 + 9*c*C18:1 + (15*t*+11*c*C18:1 / 3) + 12*c*C18:1 + 13*c*C18:1 + (14*c*C18:1+ 9*t*12*t*C18:2 / 2) + 15*c*C18:1 + 9*c*15*c*C18:2 + 12*c*15*c*18:2 + *cc*C18:2 + *cc*C18:2 + 9*c*11*t*C18:2 CLA + *ct*CLA + *cc*CLA + *tc*CLA; [6]BCFA is the sum of *iso and anteiso* FA: *iso*C14:0 + *iso*C15:0 + *anteiso*C15:0 + *iso*C16:0 + *iso*C17:0 + *anteiso*C17:0 + *iso*C18:0; [7]*n-6* PUFA is the sum of *n-6* PUFA: C18:2 *n-6* + C18:3 *n-6* GLA + C20:3 *n-6* + C20:4 *n-6*; [8]*n-3* PUFA is the sum of *n-3* PUFA: C18:3 *n-3* + C20:3 *n-3* + C20:4 *n-3* + C20:5 *n-3* + C22:4 *n-3* + C22:5 *n-3* + C22:6 *n-3*; [9]CLA = 9*c*11*t*C18:2 CLA + *ct*CLA + *cc*CLA + *tc*CLA + *tt*CLA; [10]LC *n-6* PUFA = *n-6* PUFA – LA and [10]LC *n-3* PUFA = *n-3* PUFA – ALA; [11]h/H=hypocholesterolaemic FA/hypercholesterolaemic FA. For remaining explanations see Table1.

the slaughter (1.3 and 1.5 g.100 g^{-1} FAME, respectively). When comparing with the study of Margetín *et al.* (2018), both contents of *n-6* PUFA and *n-3* PUFA for SF/SI2 lambs (7.33 and 3.01 g.100 g^{-1} FAME) were slightly lower than respective contents found for pastured IF animals (8.50 and 4.55 g.100 g^{-1} FAME).

The content of essential FAs (summed LA and ALA) was 4.64 g.100 g^{-1} FAME (BE/SI1 lambs) and 6.26 g.100 g^{-1} FAME (SF/SI2lambs), respectively. The content of CLA was 1.07 g.100 g^{-1} FAME (BE/SI1 lambs) and 1.67 g.100 g^{-1} FAME (SF/SI2 lambs), respectively. The contents of summed LA and ALA as well as CLA tend to accord both with respective values for stabled and pastured IF lambs (Margetín *et al.*, 2018). The contents of CLA, moreover, roughly agreed with Díaz *et al.* (2005) reported for male lamb crossbreds on a grass diet supplemented with hay and concentrates (0.97 and 1.05 g.100 g^{-1} FAME) and roughly agreed with the recommendation of Raes *et al.* (2004), which was less than or almost equal to 1.0 g.100 g^{-1} FAME). The findings about CLA also agreed with Daley *et al.* (2010), who showed that grass-based diets increase its amount in meat.

The ratios and indexes of FA groups that may help in assessing both nutrition value of lipids and their benefits from human health point of view are shown in Table 2. The ratios of *n-6*/*n-3* PUFA were 2.94 (BE/SI1 lambs) and 2.40 (SF/SI2 lambs); they agreed with the recommendation to be below 4, as proposed by Simpoulos (2002) and Wood *et al.* (2003). These ratios were higher than Aurosseau *et al.* (2007) reported for grazed IF male lambs, supplementally fed with hay and concentrates (1.7 and 2.2). The ratios of LC *n-6*/LC *n-3* PUFA were 1.32 (BE/SI1 lambs) and 1.18 (SF/SI2 lambs) and were higher than or almost equal as Aurosseau *et al.* (2007) reported (0.8 and 1.2). The ratios of PUFA/SFA were 0.19 (BE/SI1 lambs) and 0.30 (SF/SI2 lambs), i.e. differed from the recommendations to be above 0.7 (proposed by Raes *et al.*, 2004) or above 0.45 (proposed by Williams, 2000) or to be 0.4 at a minimum (proposed by Wood and Enser, 1997). The atherogenic index (AI) was found to be close to 1 (0.93 and 0.92, respectively) and agreed with the recommendation of Sinanoglu *et al.* (2013), who proposed this index to be 1 at a maximum. The same recommendation was proposed for thrombogenic index (TI); this was, however, slightly higher, i.e. 1.51 (BE/SI1lambs) and 1.25 (SF/SI2 lambs), respectively. The latter was almost the same as TI reported by Margetín *et al.* (2018) for pastured IF lambs (1.24). Taking into account especially AI ratios, meat of both lamb groups should be considered as healthy food consisting of beneficial FAs that may help in prevention of cardiovascular diseases (Margetín *et al.*, 2018). The ratios of hypocholesterolaemic FA/hypercholesterolaemic FA (h/H) were 1.44 (BE/SI1 lambs) and 1.58 (SF/S2 lambs), i.e. similar to h/H reported by Margetín *et al.* (2018) for pastured IF lambs (1.38).

Effect of lamb sex

The contents of FA groups, their ratios and indexes (as affected by lamb sex) are shown in Table 2. Significant differences ($P < 0.05$) between males and females were found only in the contents of *trans*-UFA, i.e. 5.10 g.100 g^{-1} FAME (males) and 5.38 g.100 g^{-1} FAME (females) and CLA, i.e. 1.28 g.100 g^{-1} FAME (males) and 1.46 g.100 g^{-1} FAME (females), following the same tendency as observed by Margetín *et al.* (2018). Regarding remaining SFA, MUFA and PUFA groups, studied ratios and indexes, no pattern was found. About half tended to be higher in males but these differences were negligible.

Coefficients of determination

The coefficients of determination (R^2), calculated for the models analysing individual fatty acids as well as their groups, various ratios and indexes in BE/SI1 and SF/SI2 lambs (ranged from 0.03 to 0.70 and from 0.15 to 0.58), were lower than R^2 reported by Margetín *et al.* (2018) for pastured and stabled IF lambs (ranged from 0.15 to 0.92 and from 0.11 to 0.90). These findings are not easy to explain; probably they are due to the fact that the models were of less precision (regarding possible factors known). Moreover, individual differences among observations tend to be higher than those accounted for systematic effects.

CONCLUSION

Analyses of fatty acids indicate that lamb meat of both breeds in the semi-intensive production systems differing in nutrition management schemes seems to be of good quality i.e. the lower contents of individual SFA (or SFA group as well) and the higher contents of individual MUFA and PUFA (or MUFA and PUFA groups as well) were found. In spite of a few exceptions, meat of lambs that were pastured (no concentrates in a diet) also in a short period before slaughter showed a slightly better composition of fatty acids. Meat of both lamb groups, however, may be recommended for human consumption.

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QUALITY EVALUATION OF FRESH GANDER SEMEN OF SLOVAK WHITE GOOSE BY CASA AND FLOW CYTOMETRY: SHORT COMMUNICATION

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ABSTRACT

In this preliminary study, the quality of fresh gander semen from the original Slovak White goose (SW) breed was analysed. Semen was collected from three (SW, n = 3) ganders into prepared sterile tubes by dorso-abdominal massage. Firstly, volume of individual gander semen was determined. Afterward, the concentration, motility parameters and sperm viability were evaluated using computer assisted semen analyser (CASA) and flow cytometry, respectively. Our results showed that the volume of individual semen samples varied from 0.05 to 0.38 ml. No significant differences in concentration and total motility of fresh semen was found among the ganders tested. However, significant differences ($P \leq 0.05$) in progressive movement of fresh semen between two males (SW2 and SW3) were observed. Moreover, differences ($P \leq 0.05$) in the percentage of apoptotic and necrotic sperm between two males (SW1 and SW3) were revealed by a flow cytometry. These preliminary data suggest that the objective assessment of fresh gander sperm motility may be an effective indicator of frozen-thawed semen quality. Therefore, regular semen assessment is required in order to preserve good-quality insemination doses from native breeds.

Key words: gander; semen; motility; CASA; flow cytometry

INTRODUCTION

Semen quality is an important factor affecting cryopreservation and fertilizing ability. Compared to mammalian or chicken semen, the data of gander semen characterisation are limited. Previous studies have demonstrated significant differences in semen characterisation among ganders (Łukaszewicz, 2002; Łukaszewicz and Kruszynski, 2003). Moreover, ganders produce a small volume of semen (0.05–1.0 ml) with a low sperm concentration (0.03– 0.8 x 10^9.ml^{-1}) and a low number of live normal cells (10–60 %). Sustained fertility in the avian female depends on its ability to store adequate viable sperm in straws and to supply the infundibulum with sufficient numbers of sperm to fertilize an ova. Only morphologically normal and vital sperm are capable of ascending through the vagina of a goose and fertilize it (Bakst *et al.*, 1994). Sperm vitality and viability are the primary determinants of fertility in domestic species (Froman and Feltmann, 1998, 2000; Froman *et al.*, 2003).

The semen analyses, essential for the study of gander quality, generally includes the evaluation of sperm motility and viability. There are many methods of assessing semen quality and estimating the fertilising potential of sperm. Common methods of semen evaluation have involved determination of the percentage of motile sperm (on a pre-warmed

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glass slide), the sperm morphology (with various staining techniques) and the concentration in using counting chamber. However, conventional semen assessment is being increasingly replaced by fluorescent staining techniques, computer assisted semen analysis (CASA) or flow cytometry.

Moreover, standard techniques of sperm viability analysis are fluorescence microscopy or flow cytometry using fluorescent staining probes. The flow cytometry technique is an automatic system able to provides precise assessment of sperm quality (Petrunkina and Harrison, 2007) because of its high sensitivity, repeatability (Christensen *et al.*, 2004; 2005) and determination of a large number (10,000) of sperm in a short period of time (Rijsselaere *et al.*, 2005). Several stains are available for evaluating cell viability and can be used alone or in combination with other dyes for the assessment of sperm quality.

In the present study, quality evaluation of Slovak White goose semen was done using CASA and flow cytometry methods. (DRAQ5, Yo-Pro-1, Sytox Green).

MATERIAL AND METHODS

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines on the care and use of laboratory animals. The treatment of the animals was approved by the Ministry of Agriculture and Rural Development of the Slovak Republic, no. SK P 28004 and Ro 1488/06-221/3a. The experiments were carried out in accordance with the Code of Ethics of the EU Directive 2010/63/EU for animal experiments.

Animals

Three clinically healthy males of breeds Slovak White goose breed (individuals marked as SW1, SW2, SW3) from 1 to 11 years, reared in a private breeding facility, were used in this experiments. All ganders were maintained in the flock and fed with wheat and oats and water given *ad libitum*.

Experimental design

Semen was collected from sexually mature gander males by dorso-abdominal massage into prepared sterile tubes twice a week in a regular manner. Samples contained urine and cell debris were removed. An aliquot taken from an individual gander semen was used for motility analysis immediately after collection. The fresh semen was diluted at the ratio of 1:4 (v:v) in a saline (sodium chloride 0.9%, B. Braun Medical Ltd. Bratislava, Slovak Republic). A part of this solution (3.4 µl) was placed on a pre-heat Standard Count Analysis Chamber Leja (depth of 20 µm) (MiniTüb, Tiefenbach, Germany) and evaluated using the CASA software under a Zeiss Axio Scope A1 microscope (Sperm Vision™; MiniTüb). Seven microscopic view fields were analysed for each sample and concentration (CON), percentage of total motile sperm (TM; motility > 5 $\mu m.s^{-1}$) and percentage of progressive motile sperm (PM; motility > 20 $\mu m.s^{-1}$) were assessed in each sample.

To assess a viability of the frozen–thawed sperm, each sample was stained fluorescently with DRAQ5 (nucleated cells) in co-stained with Yo-Pro-1 (apoptotic sperm) and Sytox Green (necrotic sperm) dyes. Samples were washed and centrifuged in a $PBS^{(-)}$ (Sigma-Aldrich, Germany) at 600 x g for 5 min and subdivided into three tubes for subsequent flow cytometric assessment of DRAQ 5-positive, DRAQ 5-positive apoptotic and DRAQ 5- positive necrotic sperm, as described below. The detection of apoptotic and necrotic sperm was performed using the specific nuclear fluorochrome Yo-Pro-1 (Molecular Probes, Switzerland) and specific nuclear fluorochome Sytox Green (Molecular Probes, Switzerland) in combination with the DRAQ5 nuclear dye (Biolegend, Germany) in order to detect only the sperm from seminal plasma.

One microliter of the Yo-Pro-1 solution (100 $\mu mol.l^{-1}$) and 0.1µl of DRAQ5 (5mM) were added to 500µl of the cell suspension to determine a proportion of apoptotic sperm. Samples were mixed and incubated in the dark at room temperature for 15 min. After incubation samples were washed in $PBS^{(-)}$ and centrifuged at 600 x g for 5 min; the supernatant was discarded. To detect portion of the necrotic sperm, firstly 0.1 µl of DRAQ5 were added to the cell suspension and incubated 15 min in the dark at room temperature. Afterwards, samples were

washed in PBS$^{(-)}$ and centrifuged at 600 x g for 5 min; the supernatant was discarded and 2.5 µl of SYTOX Green (30µM) were added to 500µl to the cell suspension and incubated for 15 min in the dark at room temperature. After the second incubation a flow cytometry assay was performed.

At least, 10,000 events were analysed for each sample. The emitted green fluorescence of YO-PRO-1, Sytox green-positive cells and the red fluorescence from DRAQ5-positive cells were recorded in the FL-1 and FL-3 channels, respectively. The different labelling patterns in bivariate analysis (e.g. Yo-Pro-1/Sytox Green) identified different sperm populations: single-positive nucleated cells (DRAQ5$^+$); double-positive apoptotic sperm (DRAQ5$^+$/Yo-Pro-1$^+$) and double-positive necrotic (DRAQ5$^+$/Sytox Green$^+$) cells.

Statistical analysis

Sperm quality among the individuals was statistically evaluated by a Tukey test using a Sigma Plot Software (Systat Software Inc., Germany). Differences at $P \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The CASA analysis and flow cytometry using fluorescence probe are commonly used for assessment of more detailed sperm characteristics in avian semen.

The present study describes the use of this technique for gander semen assessment. The quantity and quality of fresh semen depends on individual gander features as was reported in other species (Łukaszewicz *et al.*, 2002; 2004; Waberski *et al.*, 2011).

In our study, the quality parameters of fresh Slovak white gander semen were analysed. The volume of individual semen samples varied from 0.05 to 0.38 ml. No significant differences in CON and TM of fresh semen were found among the ganders tested. However, significant differences in PM of fresh semen between two males (SW2 and SW3) were observed (Table 1).

Compared with males from other poultry species such as chickens, ducks or turkeys, ganders have a relatively limited testicular development in adulthood, which results in fewer sperm produced per unit time than in other species (Łukaszewicz *et al.*, 2002). Moreover, this phenomenon may affect the reduction in motility parameters of individual ganders.

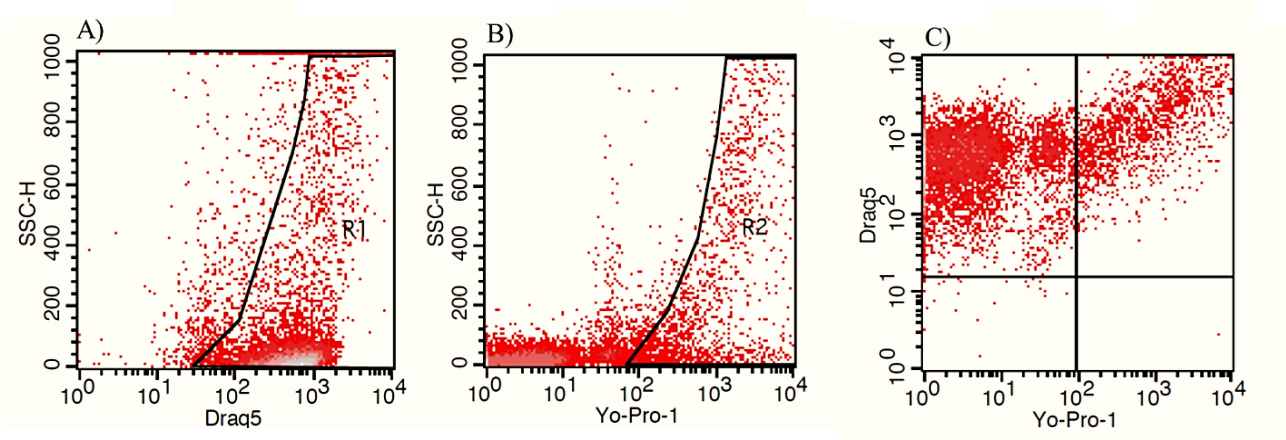

Figure 1. Representative dot plots of a flow cytometric examination of gander sperm A) Region R1 represents nuclear cells (DRAQ5$^+$) B) Region R2 represents apoptotic cells (Yo-Pro-1$^+$) C) double-positive nucleated apoptotic cells (DRAQ5+ and Yo-Pro-1$^+$). Markers of necrosis were evaluated by the same method

Table 1. The fresh gander semen characteristic

Gander	SW1	SW2	SW3
Vol (ml)	0.18 ± 0.71	0.16 ± 0.42	0.31 ± 0.23
CON (x10^9)	1.762 ± 0.68	0.96 ± 0.26	1.7 ± 0.43
TM (%)	55.37 ± 2.57	70.01 ± 3.82	55.86 ± 6.04
PM (%)	33.59 ± 3.06	49.38 ± 4.92^a	20.08 ± 0.93^b

Different superscripts indicate significant differences; ^{a} vs ^{b}, $P < 0.05$
VOL – volume; CON – concentration; TM – total motility; PM – progressive movement

We applied flow cytometry to examine proportion of apoptotic and necrotic sperm. Many authors used flow cytometry protocols to analyse specific parameters of sperm quality such as viability, apoptosis, acrosomal status, capacitation, mitochondrial membrane potential, lipid peroxidation, reactive oxygen species generation (ROS) or DNA damage (Martínez-Pastor *et al.*, 2010).

In our study the quality of the fresh Slovak white gander semen was not excellent (Table 2). We found 68.16 % of live sperm, which was lower than that obtained by Gee and Sexton (1990), who reported 92.9 % of live sperm cells in Aleutian Canada goose (*Branta canadensis leucopareia*) on eosin-nigrosine-stained slides. Other authors also showed a higher percentage of live sperm: in White Italian (*Anser anser*) gander semen – 92.2 % (Łukaszewicz, 2002), in Chinese Brown Geese – 83 % (Tai *et al.*, 2001) and in Greylag ganders – from 90.3 to 93.3 % (Łukaszewicz *et al.*, 2004).

It can be concluded that this study provides the first characteristics of Slovak white goose semen quality. Basing on the results from CASA and flow cytometry our findings revealed differences between individuals. Therefore, we suggest that the objective assessment of fresh gander sperm quality may be an effective indicator of the fertility of fresh and frozen–thawed semen from individual males. Consequently, regular semen assessment is required in order to preserve good-quality insemination doses collected from native breeds.

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Table 2. The fresh gander sperm viability

Gander	SW1	SW2	SW3
DRAQ5$^+$ (nucleated)	92.96 ± 0.41	91.25 ± 1.46	79.33 ± 3.72
DRAQ$^+$/Yo-Pro-1$^-$/ Sytox$^-$ (live)	68.16 ± 2.98	58.73 ± 3.19	25.34 ± 7.73
DRAQ$^+$/Yo-Pro-1$^+$ (apoptotic)	17.5 ± 1.35^a	21.77 ± 1.85	37.19 ± 1.43^b
DRAQ$^+$/Sytox$^+$ (necrotic)	14.34 ± 1.63^a	19.50 ± 1.34	37.46 ± 6.30^b

Different superscripts indicate significant differences; ^{a} vs ^{b}, $P < 0.05$

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