



## Comparison of meat quality from Naïma and DanBred hybrid sows mated with P-76 boars

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**ABSTRACT:** *The objective of this study was the comparative analysis of selected attributes of nutritional and technological quality of pork from Naïma and DanBred gilts mated with P-76 boars. The research was carried on 50 pigs (25 in each group) reared under the same environmental conditions and slaughtered at about 115±5.0 kg. Both analyzed crossbreeds populations were characterized by overall good nutritional and technological quality. However the loins from DanBred×P-76 compared with those from Naïma×P-76 had significantly greater glycogen and less lactate concentration in early post mortem period, higher pH at all measurement points (besides no statistical differences in pH measured 2 hours post mortem), lower thermal drip and drip loss measured 96 and 144 hours post mortem and lower lightness (except no differences measured 48 hours post mortem). Additionally loins from DanBred×P-76 contained more protein and less water. No statistical differences in IMF content between both analysed groups were noted (their average values were below 2%). Aforementioned may suggest that crossing both Naïma and DanBred hybrids with P-76 boars may not improve IMF content or increase marbling scores to the levels preferred for culinary purposes.*

**Key words:** *fatteners, nutritional value, pork, technological value.*

## Comparação da qualidade da carne de porcas híbridas Naïma e DanBred acasaladas com machos P-76

**RESUMO:** *O objetivo deste estudo foi a análise comparativa de atributos selecionados de qualidade nutricional e tecnológica de suínos provenientes de marrãs Naïma e DanBred acasaladas com machos P-76. A pesquisa foi realizada em 50 suínos (25 em cada grupo) criados nas mesmas condições ambientais e abatidos com cerca de 115 ± 5,0 kg. Ambas as populações de mestiços analisadas foram caracterizadas por uma boa qualidade nutricional e tecnológica geral. No entanto, os lombos de DanBred × P-76 em comparação com os de Naïma × P-76 tinham glicogênio significativamente maior, e menor concentração de lactato no período post mortem inicial, pH mais alto em todos os pontos de medição (além de nenhuma diferença estatística no pH medido 2 horas post mortem), menor gotejamento térmico e perda de gotejamento medido 96 e 144 horas post mortem e menor leveza (exceto nenhuma diferença medida 48 horas post mortem). Além disso, os lombos de DanBred × P-76 continham mais proteína e menos água. Não foram observadas diferenças estatísticas no conteúdo do FMI entre os dois grupos analisados (seus valores médios estavam abaixo de 2%). O resumo acima pode sugerir que o cruzamento de híbridos Naïma e DanBred com machos P-76 pode não melhorar o conteúdo de IMF ou aumentar as pontuações de marmoreio para os níveis preferidos para fins culinários.*

**Palavras-chave:** *engorda, valor nutricional, carne suína, valor tecnológico.*

## INTRODUCTION

Meat is an important component of human diet that provides proteins, vitamins and minerals required both for health and disease prevention (BIESALSKI, 2005). In Europe, the per capita meat consumption has reached 69 kg whereby pork is still most frequently consumed meat with 31.3 kg on average (EC, 2009). Nonetheless, the level of consumption is strongly associated with consumer preferences and expectations for meat (FONT-I-FURNOLS & GUERRERO, 2014). Recently,

consumers are mostly interested in lean meat but nutritious aspects, safety and quality issues (i.e. colour, drip loss, juiciness and tenderness) also plays a major role in choices and purchasing behaviour (FONT-I-FURNOLS & GUERRERO, 2014). The quality of pork is determined by variety of factors such as breed, production system, nutrition, pre-slaughter handling and slaughter procedure (WARNER et al., 2010). Over the past ten years many European countries have introduced different strategies to improve meat quality although production of high quality pork is constantly a big challenge for meat producers and

processors (VERBEKE ET AL., 2010; MORTIMER & PRZYBYLSKI, 2016).

Many pork producers use different hybrid pigs in fattening that are created by breeding companies from high performing lines. Among variety of commercial hybrid lines offered in Europe Naïma (Choice Genetics) and DanBred (DanBred International) hybrids are often used in Poland. The Naïma hybrid is maternal line created by combining of Gallia and Redone lines that merge excellent maternal performance and good carcass quality (PRZYBYLSKI et al., 2010). The Choice breeding programme assumes mating of P-76 sires with Naïma hybrids. The P-76 is a sire line issued from the Laconie (Hampshire, Piétrain and Large White in equal proportion) and Penshire (Hampshire, Large White and Duroc) lines that combines high growth potential and lean meat content with good meat quality (PRZYBYLSKI et al., 2010; LEBRET et al., 2011). DOKMANOVIC et al. (2015) and RYBARCZYK et al. (2016) indicated high lean meat content and low variability in meat quality of Naïma sows mated with P-76 boars. However the remaining majority of surveys focused on this topic are obsolete and the results may vary in comparison to quality traits of modern hybrid population. DanBred hybrid is maternal line created by crossing of Danish Landrace (L) and Danish Yorkshire (Y). DanBred hybrids, as reported by RYBARCZYK et al. (2018), SCHILD S.L.A. et al. (2020) and KASPRZYK & BOGUCKA (2020) may ensure high reproductive performance with high lean meat content and an excellent meat quality. The DanBred breeding programme assumes mating of DanBred hybrid sows (L×Y) with Duroc (D) boars. Several studies have shown the efficiency of (L×Y) × D hybrids in production of high quality pork (ZYBERT et al., 2015; CHOI et al., 2016; RYBARCZYK et al., 2018). However no data is still available regarding meat quality of DanBred hybrids mated with P-76 boars.

Because pig genotype has high impact on pork quality, the knowledge about meat quality of different pig lines and their potential to produce high quality pork is an important issue both for pig producers and meat processors. Therefore, the objective of this study was the comparative analysis of selected attributes of nutritional and technological quality of pork from two commercial hybrid lines (e.g. Naïma and DanBred) mated with P-76 boars.

## MATERIALS AND METHODS

### *Animals, slaughter and carcass treatment*

The experiment was performed according to the recommended EU Directive 2010/63/EU for

animal experiments (EU, 2010). The investigation was carried out on 50 pigs (25 gilts in each group) derived from crossing of Naïma and DanBred hybrid sows with P-76 boars. The animals were kept on the same farm in non-bedding system with an unrestricted access to water and fed *ad libitum* with a growing diet up to 60 kg (13.1 MJ metabolic energy, 170 g crude protein, 11.50 g lysine/kg) and then up to 115 kg with finished diet (12.9 MJ metabolic energy, 150 g crude protein, 9.9 g lysine/kg). Loading was performed by qualified personnel with use of sort boards. The pigs were transported to slaughterhouse (approximately 30 km) in specialised for pig transport trucks. After transport, the pigs were allowed for 2 hours rest before slaughter in lairage pens (without mixing from other transports) in accordance with density standards and constant access to water. The pigs were stunned electrically and exsanguinated in a horizontal position. Each carcass was classified according to EUROP grading system with Sydel CGM optic-needle apparatus (Sydel, France) and weighed on an electronic scale. Carcasses were chilled in chilling tunnel (-22 °C for 40 min.) and then stored at 4 °C up to 24 hours after slaughter.

### *Meat quality traits*

The quality of pork from 45 min. to 24 hours *post mortem* was evaluated directly in carcasses in the *Longissimus lumborum* (LL) behind the last rib and then in meat samples taken at 1<sup>st</sup>-4<sup>th</sup> lumbar vertebra. The samples were separated from the bone, external fat and epimysium and then stored in plastic bags at 0-4 °C. Three chops were used: one to determine drip loss and colour, second to determine pH and third for measuring of thermal drip, chemical composition and Warner-Bratzler shear force (WBSF). The pH was measured 45 min. (pH<sub>45</sub>), 2 (pH<sub>2</sub>) and 24 hours *post mortem* (pH<sub>24</sub>) directly in the right halves in the cold room and 48 (pH<sub>48</sub>), 96 (pH<sub>96</sub>) and 144 (pH<sub>144</sub>) hours *post mortem* in the laboratory using temperature-compensating pH meter pH-Star (Ingenieurbüro Matthäus, Nobitz, Germany). Electrical conductivity (EC) was recorded 2 (EC<sub>2</sub>) and 24 (EC<sub>24</sub>) hours *post mortem* directly in right halves using LF-star (Ingenieurbüro Matthäus, Nobitz, Germany). Meat colour was measured 24, 48, 96 and 144 hours *post mortem* after a 10 min. blooming period by measuring of L\* (lightness), a\* (redness) and b\* (yellowness) with a Minolta portable chroma meter (model CR 310, Minolta, Osaka, Japan) with 50 mm aperture and D65 illuminant. Drip loss (DL) was determined according to PRANGE et al. (1977) 48 (DL<sub>48</sub>), 96 (DL<sub>96</sub>), and 144 (DL<sub>144</sub>)

hours *post mortem* Water holding capacity (WHC) was determined 24 hours *post mortem* by the filter paper method according to POHJA & NINIVAARA (1957). Thermal drip was determined 48 hours *post mortem* Muscle samples (50 g) were placed in plastic bags and heated in a water bath until the internal temperature of the samples reached 72 °C. After cooling to 20 °C meat samples were reweighed. Weight loss was calculated as the difference before and after heating and expressed in percentages.

Shear force was measured using the TA-XT2 Texture Analyzer (Stable Micro Systems) with Warner-Bratzler attachment. Meat samples previously cooked to internal temperature of 72 °C and cooled to room temperature were cut out parallel to muscle fibres as cylinders with 14 mm diameter and 15 mm height. Blade speed during test was 1.5 mm/s. From the each sample, the average peak force of four cores was used for the statistical analysis. The results were presented as force per area (N/cm<sup>2</sup>).

The chemical composition of *Longissimus* muscle was determined in accordance to the official analytical methods of the AOAC (2012), water content by oven-drying 2-gram samples at 102 °C to a constant weight, crude protein content by the classic macro-Kjeldahl method, ash content by the incineration at 550 °C and lipid content by petroleum ether extraction using a Soxhlet extractor.

The mineral content (potassium K, phosphorus P, iron Fe, zinc Zn, sodium Na) was determined by atomic emission spectrometry with excitation in the inductively-coupled plasma (ICP-AES) using an Optima 3200RL camera (Perkin Elmer, USA).

#### *Muscle metabolites concentration*

The glycolytic potential (GP) was calculated according to simplified formula of MONIN & SELIER (1985) as the sum of 2[glycogen] + [lactate]. A 1g samples from *thoracic* part of *Longissimus* muscle (at the height of the last rib) were collected 45 min. and 24 hours *post mortem* and then immersed into tubes with 10 ml 0.5M HClO<sub>4</sub> and homogenized to inhibit glycolytic changes in muscles. Samples were stored at 20 °C until measurement. Glycogen content was determined by the enzymatic method according to DALRYMPLE & HAMM (1973) using amyloglucosidase derived from the yeast *Aspergillus niger*. Lactate content was determined according to BERGMEYER (1978) using lactate dehydrogenase. The glycolytic potential was expressed as μmol of lactic acid equivalent per g of fresh muscle.

#### *Statistical analysis*

Data were analysed by one-way ANOVA in STATISTICA 13.1 (StatSoft, Tulsa, OK, USA). The significance of the differences in meat quality between the two crossbreed groups was evaluated using Student's t-tests. All data were expressed as least square means and standard deviations.

## RESULTS AND DISCUSSION

#### *Carcass characteristics, chemical composition and mineral content*

In present study no statistical differences were found between hot carcass weight (HCW) and lean meat content between DanBred×P-76 and Naïma×P-76 (Table 1). RYBARCZYK et al. (2018) and RYBARCZYK et al. 2019 found slightly lower lean meat content among (L×Y)×DanAvl Duroc fatteners (57.10±1.96 % and 56.65±2.44 % respectively) that confirms high potential of P-76 boars regarding carcass quality.

The chemical composition of longissimus muscles from Naïma and DanBred hybrids mated with P-76 were presented in table 1. The Naïma crossbreed pigs had significantly (P≤0.01) higher water but lower protein, dry matter and ash content than DanBred hybrids mated with P-76 (Table 1). In contrast to our findings, no statistical differences in protein, moisture and ash were noted by KASPRZYK & BOGUCA (2020) between Naïma and DanBred hybrids. The reference data shows, that protein content in Naïma x P-76 varies between 22 and 23% while water between 72 and 74% (GRZEŚKOWIAK & BORZUTA, 2004, STRZELECKI et al., 2017) In DanBred hybrids, however mated with Danish Duroc, an average protein content varies between 20 and 22 % (RYBARCZYK et al., 2018; ZHANG et al., 2018). Total lipids is an important attribute of pork quality (HOCQUETTE et al., 2010). The amount of total lipids may positively enhance flavour, juiciness and tenderness, if they levels increase above approximately 2.5% (FERNANDEZ et al., 1999; JANKOWIAK et al., 2019). However, the consumers' acceptance of intramuscular fat (IMF) level varies in dependence of country. In Europe the acceptable amount of IMF in pork ranges from 2 to 3% (HOCQUETTE et al., 2010). There were no statistical differences (P≥0.05) in total lipids content between Naïma and DanBred hybrids mated with P-76 and its average values did not exceed 2% (Table 1). The content of intramuscular fat in Longissimus muscle may vary in dependence of breed, age, or feeding (HOCQUETTE et al., 2010). GRZEŚKOWIAK & BORZUTA

Table 1 - Carcass characteristics, chemical composition and mineral content of *Longissimus lumborum* muscle from Naïma and DanBred hybrids.

Trait	-----Group of pigs-----		P
	Danbred×P-76	Naïma×P-76	
Hot carcass weight (kg)	99.13±5.66	98.34±2.84	0.537
Lean meat content (%)	58.23±2.10	58.70±1.98	0.420
Total protein content (%)	22.99 <sup>B</sup> ±1.22	22.06 <sup>A</sup> ±0.60	0.001
Total lipids content (%)	1.61±0.20	1.54±0.42	0.460
Dry matter (%)	26.31 <sup>B</sup> ±0.89	25.14 <sup>A</sup> ±0.56	0.000
Ash (%)	1.34 <sup>B</sup> ±0.17	1.17 <sup>A</sup> ±0.12	0.000
Water (%)	73.68 <sup>A</sup> ±0.89	74.86 <sup>B</sup> ±0.56	0.000
K (g/kg)	3.76±0.73	4.02±0.20	0.086
P (g/kg)	1.02 <sup>A</sup> ±0.61	2.11 <sup>B</sup> ±0.09	0.000
Fe (mg/100g)	4.91 <sup>A</sup> ±1.21	6.83 <sup>B</sup> ±0.48	0.000
Zn (mg/kg)	13.56 <sup>A</sup> ±2.49	16.11 <sup>B</sup> ±1.45	0.000
Na (mg/kg)	4.09 <sup>b</sup> ±0.49	3.79 <sup>a</sup> ±0.28	0.013

<sup>a, b</sup> - Row means with different superscripts differ significantly by Student's t-test at  $P \leq 0.05$ .

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(2004), TRAORE et al. (2012) and PRZYBYLSKI et al. (2016) reported high levels of intramuscular fat (above 2.5%) in loins from Naïma×P-76. More recently, KASPRZYK & BOGUCA (2020) also found similarly high lipid levels in Naïma hybrids however insignificant in comparison to DanBred hybrids. In purebred Landrace and Yorkshire pigs lipid levels are often lower, below 2%. CHOI et al. (2016) found, that loins from Landrace and Yorkshire pigs contained in average 1.06% and 1.86% of lipids. Similarly low IMF levels in Landrace and Yorkshire pigs reported also ZHANG et al. (2018). Lower levels of IMF content were also observed in loins from Landrace x Yorkshire crossbreeds. In literature, an average IMF content varies from 1.14% to 1.73% (JOSELL A. et al., 2003; CHOI et al., 2016; ZHANG et al., 2018). RYBARCZYK et al. (2018) In DanBred hybrid, similarly low IMF content was reported by RYBARCZYK et al. (2018) while higher lipid levels (in average 2.5%) were found by KASPRZYK & BOGUCA (2020). In comparison to white breeds as Landrace and Yorkshire, Duroc pigs have significantly greater amount of IMF (CHOI et al., 2016; ZHANG et al., 2018). The three-way crossbreed pigs from mating Landrace x Yorkshire sows with Duroc boars, are often used in commercial pig production. Additionally, as mentioned in

introduction, DanBred hybrid programme also assumes mating of DanBred hybrid sows (L×Y) with Duroc boars. CILLA et al. (2006), SIECZKOWSKA et al. (2009), RYBARCZYK et al. (2018), ZHANG et al. (2018) reported that loins from DanBred hybrid sows mated with Duroc boars, contained about 2.5% IMF. LEBRET et al. (2011) also reported that loins from French Large White×Landrace mated with P-76 contained less intramuscular fat than those from French Large White×Landrace mated with Duroc (1.71% vs. 2.12%). PRZYBYLSKI et al. (2010) found low levels of IMF in Longissimus muscles from P-76 line. Thus, those results may partially explain low IMF content in loins of Naïma and DanBred hybrids mated with P-76 presented in this study.

Pork is an important source of minerals but their concentrations may vary in dependence of breed, age, feeding, production system or muscle (TOMOVIC et al., 2015). NIKOLIC et al. (2015) analysed the content of selected minerals in three different pork cuts from the Serbian market. They found, that in pork Longissimus muscle, Zn content ranged from 1.4 to 6.7 mg/kg. The similar Zn levels in pork loins from Serbian market reported also DJINOVIC-STOJANOVIC et al. (2017). Thus, these levels are similar to those in this study (Table 1). REN et al. (2008) found that loins from Yorkshire and

Duroc breeds contained respectively 3.7 and 3.5 g/kg K and 6.5 and 5.1 g/kg Fe without the effect of breed on minerals content. Similar to those in this study K levels in loins from (L×Y)×D also detected HAM et al. (2021). VALAITIENĖ et al. (2017) compared several pure bred and crossbreed pigs (Large White, Landrace, Yorkshire, Piétrain, Landrace×Large White, Yorkshire×Large White, Yorkshire×Piétrain and Landrace×Yorkshire) but found no differences in Zn concentration. In cited study however Landrace×Large White contained more iron than any other examined purebreds and crossbreds. Lower concentrations in Zn and Fe with in average 0.7 mg/100g Zn and 1.5 mg/100g Fe were determined by GERBER et al. (2009) in pork loins purchased from local supermarkets and butcheries in Swiss market. According to NIKOLIC et al., (2015) and GERBER et al. (2009) such variability in comparison to reference data, probably may be associated with differences in animal husbandry and applied feeding systems, or differences between breeds. As presented in table 1 Naïma hybrids had significantly ( $P \leq 0.05$ ) less sodium but contained more ( $P \leq 0.01$ ) phosphorus, iron and zinc than DanBred hybrids mated with P-76.

#### Meat quality traits

Initial glycogen concentration in muscles at the slaughter or in the early *post mortem* period

has an important role in development of pork quality. Glycogen content in muscles is determined by variability of factors such as breed, breeding strategy, feeding, pre-slaughter handling or slaughter procedures (PRZYBYLSKI et al., 2006).

An average estimates of glycogen and lactate concentrations and glycolytic potential for Naïma and DanBred hybrids mated with P-76 were shown in table 2. DanBred hybrids had significantly ( $P \leq 0.01$ ) greater GP, more glycogen (at 45 min. and 24 hours *post mortem*) and less lactate (45 min. *post mortem*) than Naïma mated with P-76. An average GP estimates although typical for normal meat were lower than those previously reported by TRAORE et al. (2012) and TARCZYŃSKI et al. (2018).

Naïma compared with DanBred hybrids had significantly lower pH at all measurement points (Table 2). The only exception was no noted statistical differences in pH measured 2 hours *post mortem*. The results may be partly explained by a higher rate of muscle glycolysis and lactate production in early *post mortem* hours in Naïma fatteners.

No statistical differences were found in  $DL_{48}$  and WHC between analysed groups. However more favourable  $DL_{96}$  ( $3.98 \pm 1.11\%$  vs.  $5.16 \pm 1.50\%$ ) and  $DL_{144}$  ( $4.97 \pm 1.21$  vs.  $6.96 \pm 1.83$ ) were noted in DanBred×P-76 than in Naïma×P-76 fatteners. Similarly to our findings no statistical differences

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in  $DL_{48}$  were noted by KASPRZYK & BOGUCA (2020) between DanBred and Naïma hybrids. Higher  $DL_{48}$  was noted by PRZYBYLSKI et al. (2016) among P-76 ( $4.68 \pm 1.96\%$ ) and by DOKMANOVIC et al. (2015) among Naïma×P-76 ( $6.30 \pm 3.01\%$ ). It is well known that drip loss is strictly associated with pH fall and extent (WARNER et al., 2010). However in aforementioned studies  $pH_{24}$  values were more favourable (respectively 0.03 and 0.25 units higher) than in present survey that may suggest other (pH-independent) factors affecting muscle drip loss. Thermal drip was statistically lower ( $P \leq 0.05$ ) in DanBred×P-76 than in Naïma×P-76 fatteners. Higher thermal drip loss noted in Naïma×P-76 may occurred due to statistically lower ( $P \leq 0.01$ ) protein content in comparison to DanBred×P-76 fatteners. According to HUFF-LONERGAN & LONERGAN (2005) the ability to maintain water in pork is strictly related to its histological structure and thus protein content. However KASPRZYK & BOGUCA (2020) noted no statistical differences in thermal drip loss between DanBred and Naïma hybrids besides its lower value in latter group.

As suggested by PRZYBYLSKI et al. (2010) average consumer's satisfaction is based mainly on visual impression (appearance) during product purchase while sensory quality and culinary usefulness is its secondary criteria. Then meat

lightness is of high importance. In present study statistically lower ( $P \leq 0.01$ )  $L^*_{24}$  and  $b^*$  but higher  $a^*_{24}$  were obtained in DanBred×P-76 than in Naïma×P-76 (Table 3). Contradictory results were noted by KASPRZYK & BOGUCA (2020) between DanBred and Naïma hybrids. Cited authors did not find however statistical differences in  $b^*_{24}$ . As suggested by KARPIESIUK et al. (2013) the higher the water content the brighter the meat colour. It is due to muscle structure which does not let the light penetrate into deeper meat layers resulting in much more intense reflection and thus higher brightness. Abovementioned is in accordance both with study of KASPRZYK & BOGUCA (2020) and present survey. Additionally higher  $L^*_{24}$  in Naïma×P-76 reported in this study probably occurred also due to lower  $pH_{24}$  as well as higher lactate content than in Naïma×P-76 (Table 2).

## CONCLUSIONS

Both Naïma and DanBred hybrids mated with P-76 boars are high lean meat crossbreeds with an overall good nutritional and technological quality. DanBred hybrids however, due to significantly better estimates for nutritional and technological quality may be more beneficial for processing and culinary purposes than Naïma×P-76 fatteners. It has

Table 3 - Least square means and standard deviation for the colour of *Longissimus lumborum* muscle from Naïma and DanBred hybrids.

Trait	-----Group of pigs-----		P
	Danbred×P-76	Naïma×P-76	
$L^*_{24}$	54.33 <sup>A</sup> ±1.84	56.78 <sup>B</sup> ±1.88	0.000
$a^*_{24}$	16.04 <sup>B</sup> ±1.08	15.06 <sup>A</sup> ±0.93	0.001
$b^*_{24}$	4.39 <sup>A</sup> ±0.54	4.93 <sup>B</sup> ±0.54	0.001
$L^*_{48}$	57.76±1.64	58.32±2.17	0.361
$a^*_{48}$	15.28±0.82	15.76±1.34	0.139
$b^*_{48}$	6.72 <sup>A</sup> ±0.88	7.83 <sup>B</sup> ±1.04	0.000
$L^*_{96}$	56.91 <sup>a</sup> ±1.78	58.31 <sup>b</sup> ±2.19	0.016
$a^*_{96}$	13.62 <sup>A</sup> ±1.42	15.42 <sup>B</sup> ±1.60	0.000
$b^*_{96}$	7.44 <sup>A</sup> ±1.66	9.54 <sup>B</sup> ±0.52	0.000
$L^*_{144}$	55.65 <sup>A</sup> ±2.36	59.19 <sup>B</sup> ±2.51	0.000
$a^*_{144}$	14.98 <sup>B</sup> ±1.08	12.60 <sup>A</sup> ±2.18	0.000
$b^*_{144}$	4.99 <sup>A</sup> ±1.00	8.57 <sup>B</sup> ±1.48	0.000

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to mentioned though that both crossbreeds did not increased total lipid content in *Longissimus lumborum* muscle to favorable level. However, all reported effects should be reconfirmed on more representative population for more accurate results.

## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study nor in data collection, analyses and interpretation.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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