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Nely Tzvetanova
Telephone.: +359 42 699446
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Effect of the addition of VemoZim F (phytase) to diets with decreased content of phosphorus on the microstructure of tibia in broiler chickens

V. Georgieva [1]*, D. Yovchev [2], A. Atanasov [3]

[1]Department of Morphology, Physiology and Animal Nutrition, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria
[2]Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria
[3]Department of Animal Husbandry, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

Abstract. *The subject of the study was to investigate the effect of the addition of different doses of the new Bulgarian enzyme preparation – VemoZim F (activity 5000 FTU/g) to wheat-corn-soyabean meal diets, with 30% decreased content of available phosphorus on the microstructure of tibia in broiler chickens. The experiment was performed with 180 one-day-old male chick broilers (Ross 308), up to the age 38 days, bred in battery cages, distributed in 5 groups, with 36 numbers in each one, equalized by body weight. The chicks from the control group obtained balanced diet, according to the requirements of the used hybrid, and these from the experimental groups, with 30% deficit of phosphorus diets (0,35, 0,32 and 0,30% in the starter, grower and finisher, respectively). The compound feed of II nd group of chicks was without addition of VemoZim F, compared to III rd, IV th and V th experimental groups, in whose diets, 150 g/t of VemoZim F was added correspondingly (recommended dose, providing 750 FTU/kg feed), 1500g/t of the investigated additive (10 times higher dose of phytase) and in the chicks from V th group 100 times more phytase than the recommended dose via the addition of 1500 g/t concentrate of phytase in VemoZim. The results of the histological analysis of tibial bone epiphysis showed, that in chickens that received 30% reduced content of available phosphorus without addition of VemoZimF, diversions in the ossification processes were observed, characteristic of phosphorus deficiency compared to the control. Remarkable differences in the chicken tibial microstructure from the control group and those that obtained 150 g/t VemoZimF (recommended dose, providing 750 FTU/kg feed) weren't found. The increased level of phytase 10 and 100 times (respectively, 7 500 and 75 000 FTU/kg feed) in the chicken diet compared to the experimental group led to different degree of structure alterations, with identical kind and localization in the epiphyseal cartilage of the tibial bones.*

Keywords: broilers, phytase, microstructure, tibial bones.

Introduction

Phosphorus is a key element which participates in the energetic metabolism of birds and it is necessary both for the normal course of assimilative processes and for the development of bone structures and maintaining the normal clinical and paraclinical status in these animals (Musavi et al., 2009). Approximately 80% of phosphorus in the animal organism participates in the mineralization of the bone structures. It increases the bone weight, especially this of the pelvic limbs, that determines the key role of this element in the osteogenesis and production of bird meat (Pintar et al., 2005).

Phytase is an enzyme which hydrolizes the phytates (Gibson and Ullah, 1990), as it liberates phosphoric acid, inositol, mineral elements, proteins, amino acids, carbohydrates and increases their availability and digestibility in monogastric animals. A lot of authors (Denbow et al., 1998; Sohail and Roland, 1999) observe the effect from the addition of microbial phytase as they report its positive influence on bone growth and mineralization. According to the same authors, phytase supports the growth of bones in length and width via transforming phytic phosphorus in available form that participates in ossification.

Qian et al. (1994) observe three zones in the epiphyseal cartilage situated consecutively from epiphisis to diaphisis in the tibial bones of broiler chickens, viz. cartilaginous, proliferative and hypertrophic. According to the same authors, the proliferative zone consists of chondrocytes, arranged in columns, with greater size than these in the cartilaginous one. The hypertrophic zone is composed of enlarged chondrocytes, being bordered by penetrating blood vessels of the metaphysis and developing ossal trabecules.

* e-mail: georgieva_vili@yahoo.com

According to Yoshida et al. (1986), with phosphorus deficiency in the diets, in the hypertrophic zone of the tibial epiphyseal cartilage in broilers, microstructure alterations, characteristic of phosphorus deficiency are found, which are connected with the increase of its length. Similar diversions from the normal histostructure of the hypertrophic zone in the epiphyseal cartilage are reported by Long et al. (1984), who find widening of its degenerative and metaepiphyseal parts.

Qian et al. (1996) investigate the influence by the addition of different qualities of phytase in turkey diets. According to their data, this enzyme influences positively the bone growth expressed in widening of cartilaginous and proliferative zones, presence of clearly traced demarcated borders among all zones of the epiphyseal cartilage. These alterations are expression of ameliorated growth, maturation, and differentiation of the chondrocyte cells and reinforcing of the mineralization processes. Milica et al. (2008) report proportional dependence between available phosphorus content and addition of phytase to the diets. According to these authors, the enzyme compensates 30 % of phosphorus deficiency in the diets. The lack of phytase and deficiency of phosphorus in the feed of broiler chickens leads to increasing the length of the hypertrophic zone. With the addition of different qualities of the enzyme phytase, independently to the decreased values of phosphorus in the diets, the histostructure of the tibial bones is similar to their normal micromorphology. The morphometric results from the studies of Qian et al. (1996) show that with increasing the phytase content the width of the cartilaginous zone grows 1,5 times, the proliferative one – 1,4, and the hypertrophic one shortens 2,4 tomes. According to Latshaw (2006), decreased quality of available phosphorus in

compound feeds of broiler chickens leads to increased penetration of blood vessels in the hypertrophic zone and difficult replacement of the chondrocytes to metaepiphyseal spongiosis.

In order to observe the conditions of feeding and breeding of broiler chickens and the necessity of presence of sufficiently available phosphorus for performance of assimilative and ossification processes, respecting the positive influence of phytase on the liberation of these from phytats, the addition of phytase is vitally important both for the rational utilization of the phytic phosphorus from plant fodder and for the economy of inorganic phosphorus additives and ecologization of the enviroment. The aim of the present study is to examine the effect of addition of different doses of the new Bulgarian enzyme additive - *VemoZimF* (phytase) to diets with decreased nonphytic phosphorus content on the microstructure of broiler chicken tibia.

Material and methods

One hundred and eighty one-day-old male broiler chickens (Ross 308) bred in battery cages, were distributed in 5 groups (control and 4 experimental), 36 numbers in each one, equalized by body weight, each group was with 3 repetitions. The duration of the experiment was 38 days. The chickens were bred at 24-hours electric lighting, and they obtained food and water *ad libitum.* During the experimental period the microclimatic conditions were supported in accordance with the regulatory parameters prescribed for Ross 308.

Diets. The starter, grower and finisher wheat-corn-soybean meal diets used in the experiment were factory made, balanced to content of energy and compound feeds according to the requirements of the hybrid, but with 30% lower content of available phosphorus (0,35, 0,32 and 0,30%, respectively, in the starter, grower and finisher). The level of available phosphorus in the diets of the control (Ist group) was increased to the requirements of the hybrid, for starter/grower/finisher (0,5, 0,45 and 0,42%, respectively), by addition of monocalcium phosphate, and the level of calcium in the starter, grower and finisher feeds in the experimental groups was equalized with the control one (1,15, 0,99 and 0,93 %, respectively) by addition of limestone. The chickens from IInd experimental group obtained diets with 30% reduced level of available phosphorus, and the ones from the rest three groups were fed with the same diet, enriched with enzyme additive *VemoZim F*, containing microbial phytase (activity 5000 FTU/g). The IIIrd experimental group obtained 150 g/t (recommended dose, providing 750 FTU/kg feed) from *VemoZimF* and IVth and Vth groups – 1500g/t (10 times higher dose of phytase) and 1500 g/t concentrate of the enzyme (providing 100 times more phytase per1 kilo of feed), respectively. At the end of the experimental period 5 chickens with mean body weight were taken from each group whose left tibial bones were preparated post mortem for studying the influence of the applied feeding on the microstructure of the same bones.

The histological investigation was performed on bio trials, obtained from epiphysis of tibial bones. The studied material was fixed in 10% neutral formalin. The fixed elements were once washed in water and decalcified in 10% nitrogen acid for 12 days, after that they were dehydrated in ascending alcohol row and embedded in paraffin. Histological cuts with thickness of 8 μм were obtained from them, afterwards they were stained with hematoxilin – eosin via a method modified by us Volkova and Eletski, (1976). Histomorphometric study of the growimg zones of the epiphyseal cartilage was made by the method of Avtandilov (1990).

Results and discussion

The histological analysis of bone material from the tibial proximal extremity obtained from Ist (control) group demonstrated microstructure features characteristic of the normal structure of the chicken bones, i.e. a presence of three distinctly differentiated and

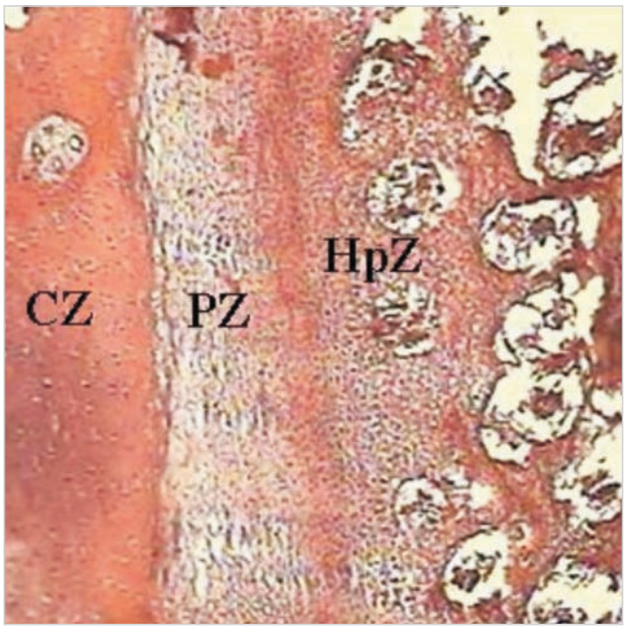

Figure 1. Normal histostrucure of epiphyseal cartilage with presence of three distinctly differentiated zones: cartilaginous (CZ), proliferative (PZ), hypertrophic (HpZ).

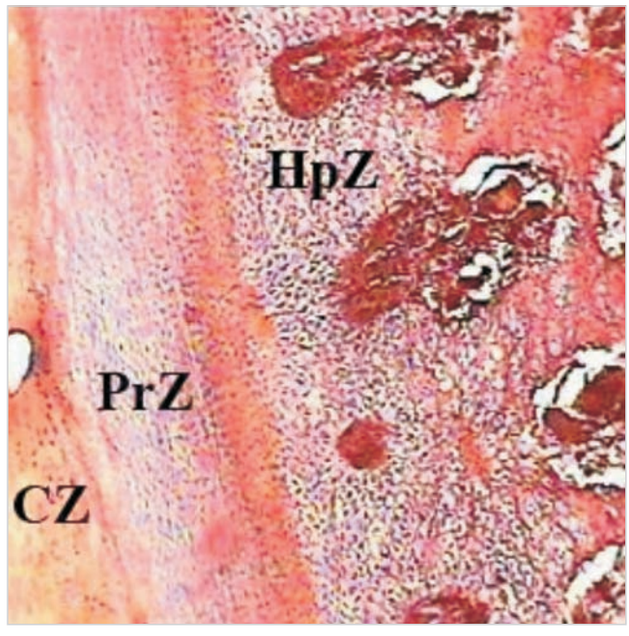

Figure 2. Alteration of epiphyseal cartilage histostrucure at phosphorus deficiency. Narrowing the cartilaginous (CZ) and proliferative (PrZ) zones, widening of the hypertrophic one (HpZ).

Figure 3. Alteration of epiphiseal cartilage histostructure, at addition of recommended doze *VemoZim F* (fitase) and 30% deficiency of available phosphorus. Presence of distinctly differentiated cartilaginous (CZ) and proliferative (PZ) zones and narrowing of the hypertrophic one (HpZ).

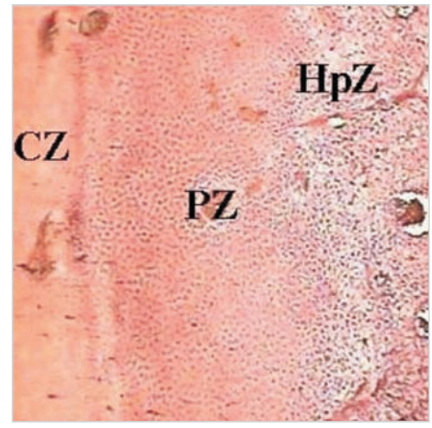

Figure 4. Alteration of epiphyseal cartilage histostructure, at addition of 10 times increased dose of VemoZim F and 30% deficiency of available phosphorus. Widened cartilaginous (CZ) and proliferative (PZ) zones and narrowing of the hypertrophic one (HpZ).

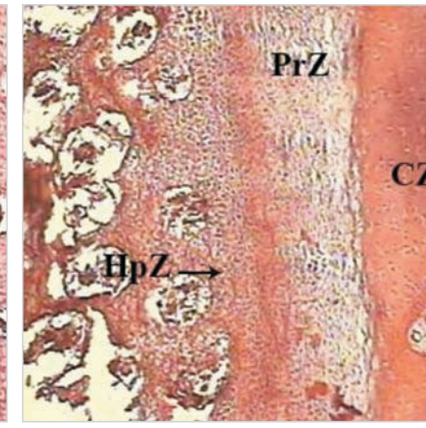

Figure 5. Alteration of epiphiseal cartilage histostructure, at addition of 100 times increased dose of VemoZim F and 30% deficiency of available phosphorus. Widened cartilaginous (CZ) and proliferative (PrZ) zones and narrowing of the hypertrophic one (HpZ).

consecutively situated zones in the epiphyseal cartilage – cartilaginous, proliferative and hypertrophic. The chondrocytes in the first zone were small and distinctly differentiated compared to the same cellular structures in the proliferative and hypertrophic zones that confirmed the attitude of Qian et al. (1994) about the presence of zonality in the epiphiseal carilage microstructure in these birds. Highly marked demarcation borders were observed between the separate parts which is a proof about ossification processes that have normally taken place (Figure 1).

The results from the investigated bio trials, obtained from broiler chicken epiphyses from the IInd experimental group showed microstructure alterations, characteristic of phosphorus deficiency and disturbed mineralization processes, that confirmed the attitude of many authors (Pintar et al., 2005; Musavi et al., 2009) about the key role of available phosphorus in the growth and development of bone elements. These changes came down to increase the width of the hypertrophic zone, widening of the degenerative and metaepiphyseal parts of these and enhanced penetration of blood vessels to its border with the proliferative one that corresponds to alterations in the microstructure of tibial bone epiphyseal cartilage observed by other authors in turkeys and broiler chickens characteristic of phosphorus deficiency (Long et al., 1984; Yoshida et al., 1986; Milica et al., 2008) (Figure 2).

The addition of the recommended dose for broiler chickens of enzyme premix *VemoZim F* (phytase) - 150 g/t to the diet of the IIIrd experimental group independently of 30% decreased content of available phosphorus resulted in alterations in the epiphyseal morphostrucures which are observed in normal mineralization processes. They were manifested in decrease of the width of the hypertrophic zone, increase the sizes of cartilaginous and proliferative ones, in accordance with the findings of Qian et al. (1996). The borders between the described zones were distinguishable, like the histological findings, referring to the control group. The obtained results corresponded with data by a lot of authors (Gibson and Ullah, 1990; Denbow et al., 1998; Sohail and Roland, 1999) about the key role of the enzyme phytase in turning phosphorus in available form for bird metabolism and its indirect positive influence on the bone growth (Figure 3).

The increased level of added phytase, 10 and 100 times, respectively, in the diets of IVth and Vth groups of broiler chickens, led to alterations in the same point, observed in IIIrd experimental group, but in intensified degree, without negative influence on the

Table 1. Alteration in the width of the three zones of tibial epiphiseal cartilage in broiler chickens, μm (Mean values ±SD, n=5)

Group *VemoZim F* (phytase) (FU/kg)	1	2 ** 	3 ** 5000	4 ** 50 000	5 ** 500 000
Cartilaginous zone (40 x)	236,34±25,59	200,79±19,39	220,66 ±17,18	224,42±20,46	230,94±27,64
Proliferative zone (100 x)	106,76±11,34	78,89*±9,85	102,56*±12,14	103,78±13,86	107,78±12,00
Hypertrophic zone (100 x)	82,37±8,37	161,36*±20,22	110,34*±18,67	100,08±15,18	88,10±14,37

*P<0.01 between the group without and with addition of recommended dose of VemoZim F (phytase)
** 30% decreased quantity of available phosphorus in the diets

bone structure, that is confirmed by the results of Milica et al. (2008). They were reduced to quantitative changes in the cartilaginous, proliferative and hypertrophic zones, as the first two were widened, and the hypertrophic one was considerably narrowed.

The finding from our results verified the morphometric data of Qian et al. (1996) about the important influence of phytase on mineralization processes (Figures 4 and 5).

The results from our morphometric investigations proved that the addition of the recommended dose of VemoZim F in the diets of broiler chickens, compensates 30% phosphorus deficiency that confirmed the attitude of many authors (Qian et al., 1996; Milica et al., 2008), about the effective influence of the enzyme phytase on the liberation of phosphorus from phytates and its inclusion in the mineralization processes (Table 1).

Conclusion

The results from our histological and morphometric studies proved that the addition of the recommended dose of VemoZim F (phytase) in the diets of broiler chickens compensates 30% phosphorus deficiency that is demonstrated with alterations in the lengths of the cartilaginous, proliferative and hypertrophic zones, as the first two are elongated, and the last is widened considerably. These changes are manifested with presence of normally occurring processes of mineralization. The repeated increase of VemoZim F - 10 and 100 times led to alterations in the histostructure of the epiphyseal cartilage observed in IIIrd experimental group (obtained recommended dose of VemoZim F – 750 FTU/ kg feed), but in intensified degree, without negative influence on the bone structure.

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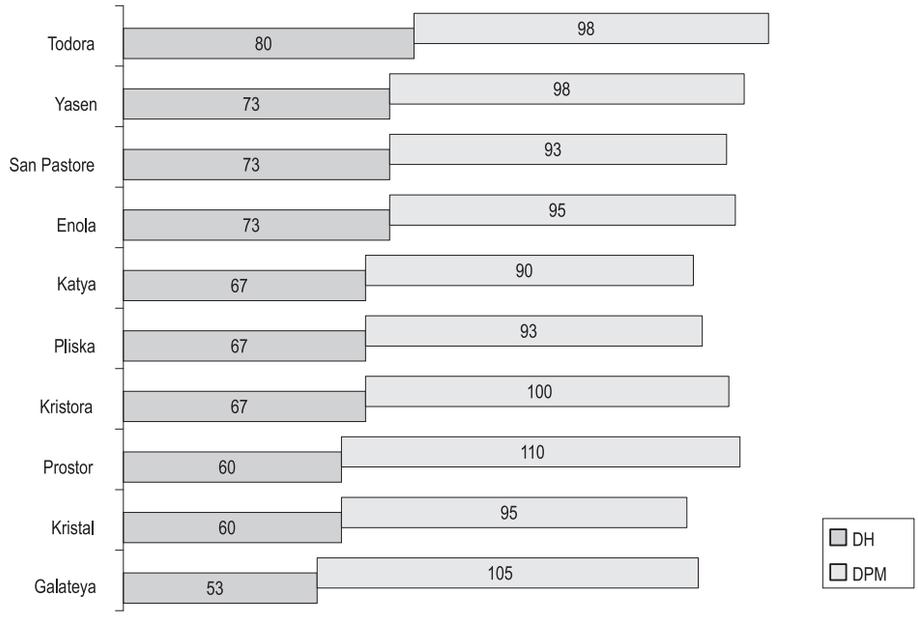

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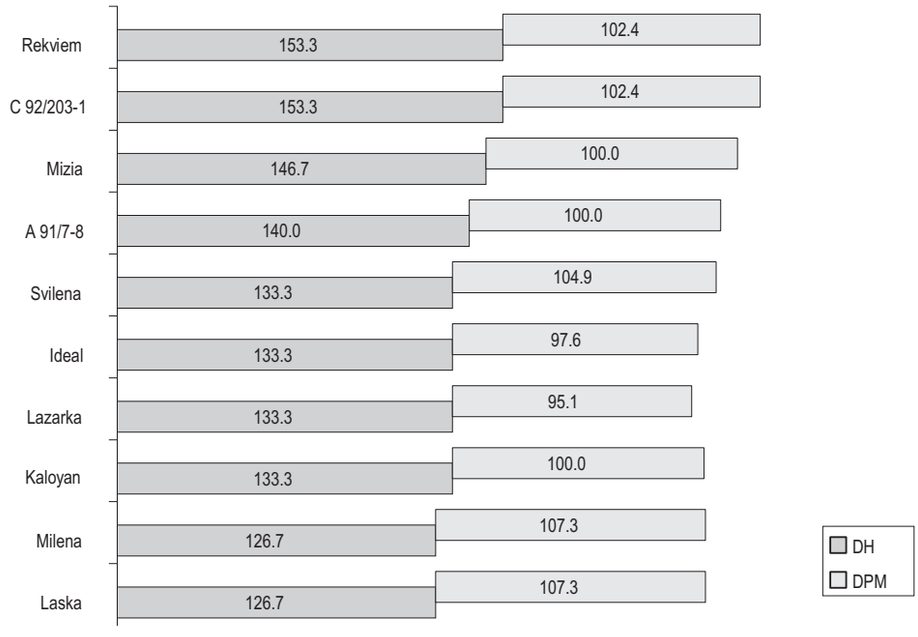

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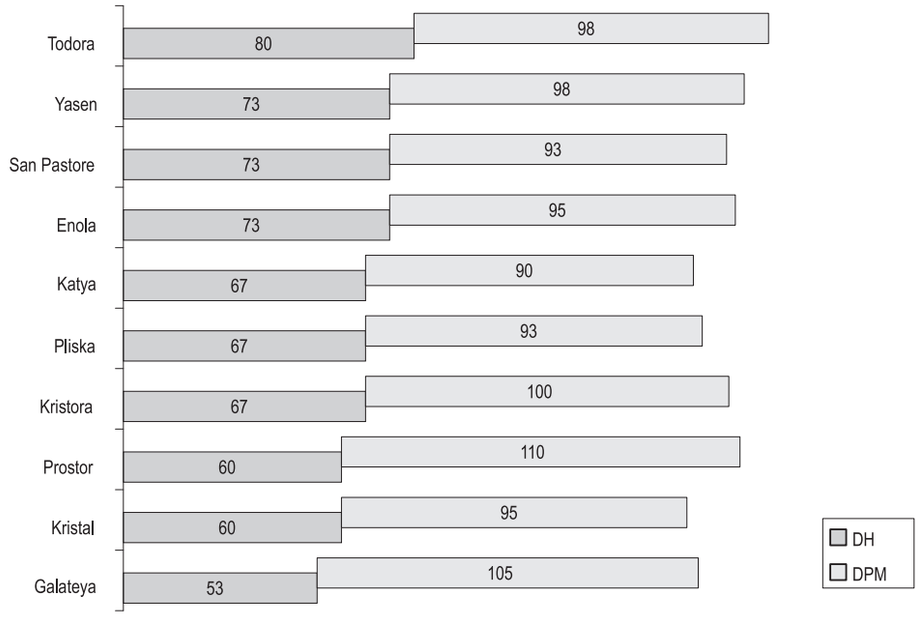

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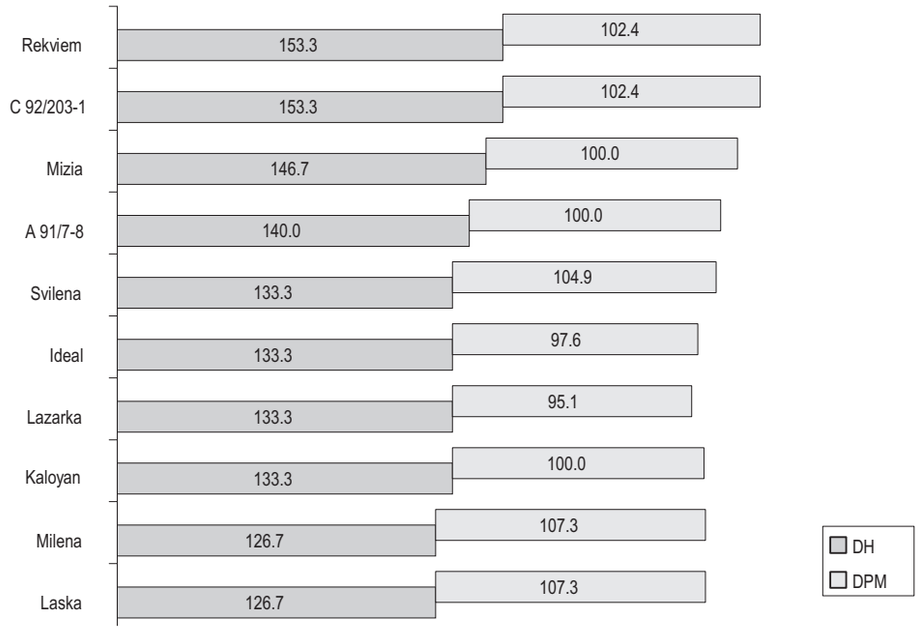

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