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Investigation of the possibilities of using PON1 enzyme activity in animal improvement

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Abstract: In this study, the activity of paraoxonase 1 (PON1) in various cattle races in different age groups was investigated. PON1 is capable of hydrolyzing organophosphate agents of pesticides that are amongst the most significant stress sources for farm animals. PON1 can also hydrolyse nerve gases and can protect the organism against lipid peroxide formations that occur as a result of oxidation of LDL. The cattle races in the study were separated into 3different age groups of young (0 to6 months), middle-age (6 months to 4 years) and old (4 years of age and above), and 5 mL of blood sample was collected from each animal to analyse and determine their PON1 activities. The statistical analyses were then performed. Based on the knowledge that the animals with high PON1 enzyme activity would have higher adaptation capabilities for regions that are being sprayed with pesticides compared to animals with low PON1 enzyme activity, the possibility of using PON1 activity tests as a potential precharacterization study for marker assisted selection (MAS) was investigated. The results have revealed that PON1 enzyme activity could be used as a biomarker for determination of pesticide resistance.

Key words: Biomarker, PON1 activity, animal improvement

1. Introduction

Due to the limited amount of agricultural production area available, animals are being rehabilitated to get the maximum performance from each animal with expectations to be able to meet the increasing nourishment needs of humanity [1]. Fast and accurate estimations of existing animal husbandry data are essential to be able to increase future product performances in agriculture and livestock practices [2]. Since the 1990s, phenotype and parent information of the animals have been used to estimate their breeding values, and all genetic progress was achieved based on the phenotypic selection of animals for breeding purposes [3].

Genetic improvements of efficiency characters of farm animals take place very slowly due to (a) certain efficiency features being only measurable for 1of the sexes, (b) expression of numerous genes being obtained by the sum of their effects, and (c) major impact of environmental effects on the quantitative characters. This situation reduces the accuracy of genetic evaluations as well. Furthermore, generation range gets longer and the rate of genetic improvement slows down due to the fact that productivity qualifications can only be measured on adult animals [4].

The possibility of utilizing genome analysis to provide faster genetic progress on productivity characters has been investigated in recent years. Genome analyses are performed in 3major types as structural, functional, and comparative analyses. Functional genomics studies, biological functions of genes, and the formations and products thereof [5].

Genes in a cell give directions to the interior systems of the cell to perform their functions. Various biochemical events occur in the cell as a result of these directions. Metabolites arise from these biochemical events. A phenotype for the aforementioned features can be presented by revealing their relationship with the diseases, efficiencies, and their adaptation.

Factors that affect the appearance of metabolites must be fully determined in order to be able to use them in selection studies. The factors that affect metabolites are generally the species, race, age, productivity period, and health conditions of the animals.

Metabolomics can be used as biomarkers for some diseases. The relationships between the metabolites and the productivity performances of animals, their adaptation capacities, and their resistance to diseases were identified, and the potential use of metabolites on selection studies was

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investigated. Productivity performances of farm animals are generally controlled by the genetic configurations and the environmental factors. Animal breeders try to provide suitable environmental conditions for animals to get the maximum amount of yields from them. While certain environmental factors can be controlled, others cannot be [2]. This is particularly important considering the fact that each and every environmental factor causes a certain level of stress on animals [1].

One of the important stress sources for animals is the pesticide use in the fight against agricultural pests. These pesticides enter the metabolisms of the animals either directly or through their feeds. It has been estimated that only 0.015% –6% of the applied pesticide reaches the targeted pests, whereas the remaining 94%–99.9% just contaminate the environment, the soil, and other untargeted organisms [6].

Harmful effects of pesticides on organisms can be classified into 2groups as acute and chronic effects. Acute reactions can vary from slight allergies to death. Chronic effects include; neurotoxicity, behavioural disorders, reduced breeding and fertility, and mutagenic, teratogenetic, and carcinogenic effects [7,8].

Paraoxon is a catabolic output of the parathion compound, which in turn is a pesticide used to increase agricultural productivity. Paraoxon initially inhibits the activities of acetylcholine esterase and certain other enzymes that play important roles in nerve impulse transmitting. That being said, organisms have already developed defense systems for most of these effects. One such defence mechanism is the paraoxonase (PON) enzyme. This enzyme eliminates the harmful effects of paraoxon by hydrolysing it [9]. PON enzyme also has a certain degree of antioxidant activity [10,11]. It has been suggested that on cattle, factors like race, age, productivity period, and health issues can cause changes on PON1 enzyme activity [12,13,14].

By presenting PON1 enzyme activities of cattle with different race and age properties in this study and considering the data obtained from previous studies regarding the higher resistance profiles of some animals with high PON1 activity against certain diseases and organophosphate poisoning, this study aims to investigate the opportunities for performing genomic analyses using functional genomics method to determine adaptation capabilities and disease resistance of the animals, and provide a precharacterization study for future marker-assisted selection (MAS) studies.

2. Materials and methods

2.1. Material

Holstein, Jersey, and Simmental race animals were used as study materials, considering their high productivity performance and field utilization. Three age groups were created for each race as young (0 to 6 months), middle age (6 months to 4 years) and old (4 years and above). A total of 54 cattle were used, each group having 6 cattle. 5 mL blood was taken from the tail veins (V. coccygea) of young cattle, and from the jugular vein (V. jugulars) of others.

2.2. Method

Blood samples were centrifuged with 5000 rpm in +4 °C for 10 min, after which the serum collected on the top was collected into falcon tubes using a dropper. These were then kept in −70 °C until the activities were measured.

Buffer solution (850 µL), 100 µL paraoxon solution, and 100 µL serum were added together rapidly, and absorbance taking place in 1 min in 37 °C on 412 nm was measured. The same process was repeated without the enzyme and the difference was determined as the unit enzyme performance. One unit of paraoxonase was determined as nmol of p-nitrophenol occurring in a single minute.

In the statistical analysis, the comparisons between race and age groups were performed using Univariate Analysis of Variance method. Duncan test was utilized as the multiple-comparison test. SPSS 23 package software (IBM Corp., Armonk, NY, USA) was used to perform the statistical analysis.

3. Results

3.1. PON1 activity in young cattle group

Holstein, Simmental, and Jersey young cattle between 0 and 6 months of age, and their PON1 enzyme activity values are presented in Table 1 with average value, standard deviation, standard error, and minimum and maximum values.

When Table 1 is inspected, it can be seen that the PON1 enzyme activities of Holstein, Jersey, and Simmental races were determined as 114.24, 450.80, and 482.53, respectively. Statistical difference between the mentioned groups was verified to be significant ($P < 0.01$). It can be understood from in multiple comparisons that, the reason for the variation is raised from the lower PON1 enzyme activity of Holstein race compared to the other races.

3.2. PON1 activity in middle age cattle groups

Middle age cattle groups consisted of animals between 6 months and 4 years of age, and their data are presented in Table 2 as an average value, standard deviation, standard error, and minimum and maximum values.

When Table 2 is inspected, it can be observed that middle age Holstein animals had an average activity of 19.60, while Jerseys had an average of 264.73, and Simmental had an average of 200.25. Although the PON1 activity of Jersey race in the middle age group was higher than the other groups, this difference was determined to be statistically insignificant.

Table 1. PON1 activity in young age groups (U/L).

0 to 6 months	Average	Standard deviation	Standard error	Minimum	Maximum
Holstein	114.24[b]	26.19	10.69	63.64	133.33
Simmental	482.53[a]	148.06	34.90	236.84	702.20
Jersey	450.80[a]	246.17	71.06	213.41	926.67
F value	10.19**				

**P < 0.01, [a, b] Values with different letters in the same column were found to be different from each other (P < 0.01).

Table 2. PON1 enzyme activity in middle age groups (U/L).

6 months to 4 years	Average	Standard deviation	Standard error	Minimum	Maximum
Holstein	193.60	86.44	35.29	91.78	347.14
Simmental	200.25	97.29	22.93	86.90	393.88
Jersey	264.73	76.69	22.14	134.43	387.50
F value	2.208 [-]				

[-] P > 0.05.

3.3. PON1 activity in old age cattle groups

Old age was determined as 4 years and above. Values are presented in Table 3 as average, standard deviation, standard error, minimum and maximum.

As can be seen in Table 3, the average activity value for the old Holstein group was found to be 125.41, whereas old Jersey group had an average value of 270.25, and old Simmental group had an average value of 104.53. PON1 activity of old Jersey group was found to be higher compared to the others and this difference was statistically significant (P < 0.01).

3.4. PON1 activity according to age groups

Age groups compared to each other, regardless of race and values are given in Table 4.

As Table 4 is inspected, it can be seen that PON1 activity values of young groups had an average of 349.19, while middle age group had an average of 219.53, and old age group had an average of 166.73. Although the average value for young cattle was found to be higher compared to the other age groups, multiple comparisons revealed that the difference was caused by the low activity value of PON1 in the old age group.

3.5. PON1 activity according to race groups

Values of PON1 activity based on race are presented in Table 5 with average value, standard deviation, standard error, and minimum and maximum values.

Average values for Holstein, Simmental, and Jersey races are shown in Table 5. The average value for the Holstein race was found as 144.42, whereas the average value for the Simmental race was 262.44, and the average for the Jersey race was 328.59. As can be seen, the Jersey race has an advantage in terms of average PON1 activity over the other races. The difference between race groups was found to be statistically significant (P < 0.01).

3.6. Correlation and regression values between age and PON1 activity

Correlation ratio between PON1 activity and age of cattle with different ages were found as -0.408. This value is considered to be moderately negative and is statistically significant with a level of P < 0.01. According to this result, as age increases, PON1 activity decreases in a significant manner.

Regression ratio between different ages and PON1 activity was found as 0.430. In this study, the PON1 enzyme activity level was found to decrease by –91.229 for each 1 year of age increase, and the regression ratio was found to be statistically significant.

The age regression equation of PON1 = 427.608–91.229* was obtained to describe the relationship between age and PON1 enzyme activity. Furthermore, R^2 (determination ratio) was found to be 0.185. This value indicates that 18.5% of PON1 enzyme activity change was based on the age of the cattle.

Table 3. PON1 activity values in old cattle races (U/L).

4 years and above	Average	Standard deviation	Standard error	Minimum	Maximum
Holstein	125.41[b]	47.32	19.32	71.43	202,44
Simmental	104.53[b]	14.43	3.40	88.24	133,33
Jersey	270.25[a]	128.03	36.96	87.71	441,67
F value	17.601**				

** P < 0,01, [a, b] Values with different letters in the same column were found to be different from each other (P < 0.01).

Table 4. PON1 activity according to age groups (U / L).

Age groups	Average	Standard deviation	Standard error	Minimum	Maximum
0 to 6 months	349.19[a]	237.73	56.03	63.64	926.67
6 months to 4 years	219.53[a]	91.35	21.53	86.90	393.88
4 years and above	166.73[b]	108.54	25.58	71.43	441.67
Chi-square	8.937**				

** P < 0.01, [a, b] Values with different letters in the same column were found to be different from each other (P < 0.01).

Table 5. PON1 activity based on race groups (U/L).

Race groups	Average	Standard deviation	Standard error	Minimum	Maximum
Holstein	144.42[b]	66.03	15.56	63.64	347.14
Simmental	262.44[ab]	194.41	45.82	86.90	702.20
Jersey	328.59[a]	186.34	43.92	87.71	926.67
Chi-square	14.07**				

** P < 0.01, [a, b] Values with different letters in the same column were found to be different from each other (P < 0.01).

4. Discussions

Numerous studies about organophosphate toxicity or disease sensitivity were presented only as polymerase chain reaction (PCR) based nucleotide polymorphism studies (Q192R, L55M, C-108T). Although this analysis method is more informative, direct measurement of PON1 enzyme activity as functional genomics analysis also indicates the amount of all polymorphisms that may influence the activity. On the other hand, enzyme determination with paraoxon and diazoxon PON1 enzyme substrates can be carried out with higher efficiency [15,16]. As the PON1 enzyme activity increases, its protective effect against organophosphate poisoning also increases. It is thereby very important to determine the PON1 enzyme activity in a given population [15,17,18]. Even though PON1 and SNP amounts can be determined for each individual animal, these alone are not enough to provide accurate information regarding the PON1 activity and its effectiveness on that particular individual animal. In other words; an enzymatic analysis with 2 substrates is required to reveal exact PON1 levels and its functional efficiency [19]. Factors affecting PON1 enzyme activity should be revealed to be able to use the results of this study as biomarkers. PON1 enzyme activity is influenced by factors like race, species, sex, health condition, and animal welfare [20,21].

The effects of race and age on the PON1 enzyme activity of cattle were determined in this study. Average PON1 activity value for the Holstein race was determined as 144.42. This value is higher compared to Holstein race dry (97.17) and the postpartum period (83.17) average PON1 activities and as well as the peak lactation period's (90.93) determined by Kulka et al. [22]. On the other hand, this value is lower compared to Holstein-Friesian (178.69), Polish Red (168.54) and Norwegian (145.8) race average values determined by Erzengin et al. [14] and Kulka et al. [23]. On the other hand, the average PON1 enzyme activity determined in this study (144.42) was similar to the average Holstein race value (152.29) determined by Turk et al. [24], while it was lower compared to pregnant Holstein cows in first 3 months of pregnancy (283), 3 to 6 months of pregnancy (257), intramammary infusion (216) pre-puerperal in nonpregnancy (223), late puerperal (189), and peak lactation (329) values determined in the same study [24].

PON1 enzyme activity average values in Simmental and Jersey races were found to be 262.44 and 328.59, respectively. PON1 enzyme activity values for these races were not found in literature searches. However, Turk et al. [24] have determined similar values for pregnant cows of the Holstein race (first 3 months of pregnancy (283), 3 to 6 months of pregnancy (257), and peak lactation (329)). The researchers also reported higher values than the aforementioned averages for intramammary infusion period (216) and nonpregnant cows (pre-puerperal in nonpregnancy (223), late puerperal (189)).

It is believed that Simmental and Jersey race cows have higher adaptation capabilities compared to the Holstein race based on the fact that they can be raised in even average-quality pastures, which in turn may depend on higher average PON1 enzyme activity.

The result of decreasing PON1 enzyme activity with age as was shown in this study was found to be similar to another study performed for 17–19-month Heifer and 3–4-year-old Holstein cows by Antonic-Svetina et al. [12]. Similar results were also reported for Wistar rats in which the researchers report a PON1 enzyme activity change due to age [25], and a study performed by Taha et al. [26] on female camels.

According to all this information, it has been concluded that necessary preconditions were fulfilled for using average PON1 enzyme activity value of cattle races as a biomarker towards pesticide resistance. Although there are other types of studies for this particular property, no study was found in the literature to use it as a biomarker for cattle. It was shown in the research of Wan-Fen et al. [27] that PON1 enzyme activity could be used as a biomarker on humans for pesticide resistance [27].

In a study performed on Drosophila melanogaster flies which lack PON enzymes as a species, transgenic flies with the capability of producing PON1 enzyme were obtained first, and the effects of this change on Pseudomonas aeruginosa (a gram - bacteria) virulence on the flies were investigated. It was determined that the transgenic PON1 enzyme protected flies from the deadly effects of Pseudomonas aeruginosa and helped them resist to organophosphate toxicity [28].

As the functional genomics practices used in genomic selection studies become more practical and feasible, researchers are beginning to understand the importance of functional genomics and are performing more studies on this subject. The results of this hereby study have revealed the necessity for similar studies, as well as the fact that PON1 enzyme activity could be used as a biomarker on cattle for determined resistance against pesticides.

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References

1. Akçapınar H, Özbeyaz C. Hayvan Yetiştiriciliği Temel Bilgileri. Ankara, Turkey: Nadir Kitap; 1999 (in Turkish).
2. Aksoy AR. Hayvan Islahı Ders Notları. Kars, Turkey: Kafkas Üniversitesi Veteriner Fakültesi; 2003 (in Turkish).
3. Montaldo HH, Meza-Herrera CA. Use of molecular markers and major genes in the genetic improvement of livestock Electron. Journal of Biotechnology 1998; 1: 83-89.
4. Lara M, Gama L, Bufarah G, Sereno J, Celegato E et al. Genetic polymorphisms at the k-casein in the pantaneiro cattle. Archivos de Zootecnia 2002; 51: 99-105.
5. Bal SH, Budak F. Genomik proteomik kavramlarına genel bakış ve uygulama alanları. Uludağ Üniversitesi Tıp Fakültesi Dergisi 2013; 39: 65-69 (in Turkish with an abstract in English).
6. Tiryaki O, Temur C. The fate of pesticide in the environment. Journal of Biological and Enviromental Sciences 2010; 4: 29-38.
7. Tarakçı Ü, Türel İ. Halk sağlığı amaçlı kullanılan pestisitlerin (Biyosidal) güvenilirlik standartlarının karşılaştırılması. YYU Veteriner Fakültesi Dergisi 2009; 20: 11-18 (in Turkish with an abstract in English).

8. Nougadere A, Merlo M, Heraud F, Rety J, Truchot E et al. How dietary risk assessment can guide risk management and food monitoring programmes: The approach and results of the French Observatory on Pesticide Residues (ANSES/ORP). Food Control 2014; 41: 32-48.

9. Costa L, Li W, Richter R, Shih D, Lusis A et al. The role of paraoxonase (PON1) in the detoxication of organophosphates and its human polmorphisms. Chemico Biological Interactions 1999; 429: 119-120.

10. Graham A, Hassall DG, Rafique S, Owen JS. Evidence for a paraoxonase-independent inhibition of low density lipoprotein oxidation by high-density lipoprotein. Atherosclerosis 1997; 135: 193-204.

11. Aviram M, Hardak E, Vaya J, Mahmood S. Human serum paraoxonase (PON1) Q and R electively decrease lipid peroxides in human coronary and carotid lesions. Circulation 1999; 101: 2510-17.

12. Antonic-Svetina M, Turk R, Svetina A, Geres D, Rekic B et al. Lipid statusparaoxonase-1 activity and metabolic parametres in serum of heifers and lactatinh cows related to oxidative stress. Research in Veterinary Science 2011; 90: 298-300.

13. Giordino A, Veronesi M, Rossi G, Pezzia F, Probo M et al. Serum paraoxonase-1 activity in neonatal calves: Age related variations and comparison between healthy and sick animals. Veterinary Journal 2013; 197: 499-501.

14. Erzengin M, Demir D, Arslan M, Sinan S. Purification and characterization of paraoxonase 1 (PON1) from Swiss Black Holstein and Montofon bovines. Applied Biochemistry Biotechnology 2014; 173: 1597-1660.

15. Richter R, Clement E. Determination of paraoxonase (PON1) status requires more than genotyping. Pharmacogenetics 1999; 9: 745-53.

16. Richter RJ, Jampsa RL, Jarvik GP, Costa LG, Furlong CE. Determination of paraoxonase 1 status and genotypes at specific polymorphic sites. Current Protocols in Toxicology 2004. doi: 10.1002/0471140856.tx0412s19

17. Eckerson HW, Romson J, Wyte C, La Du B. The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. American Journal of Human Genetics 1983; 35(2): 214-227.

18. Davies HG, Richter RJ, Keifer M, Broomfield CA, Sowalla J et al. The effect of the human serum paraxonase polmorphism is reversed with diazoxon soman and sarin. Nature Genetics 1996; 14: 334-336.

19. Costa L, Cole T, Vitalone A, Furlong C. Measurement of paraoxonase (PON1) status as a potential biomarker of susceptibility to organophosphate toxicity. Clinica Chimica Acta 2005; 352: 37-47.

20. Hillel J, Dunninton E, Siegel P. DNA markers in poultry breeding and genetic analyses. Poultry Science 1992; 4: 169-186.

21. Nicholas F. Introduction to Veterinary Genetics. Oxford, UK: Oxford University Press; 1996.

22. Kulka M, Beltowski J, Klucinski W, Orlowska M, Kolodziejska J et al. Serum paraoxonase-1 activity of dairy Holstein-Fresian cows in different lactation stages–preliminary study. Polish Journal of Veterinary Sciences 2014; 17: 143-147.

23. Kulka M, Kolodziejska-Lesisz J, Klucinski W. Serum paraoxonase (PON1) activity and lipid metabolism parametres changes in different production cycle periods of holstein-friesion an Polish red and Norwegian breeds. Polish Journal of Veterinary Sciences 2016; 19: 165-73.

24. Turk R, Juretic D, Geres D, Svetina A, Turk N et al. Influence of oxidative stress and metabolic adaptation on PON1 activity and MDA level in transition dairy cows. Animal Reproduction Science 2008; 108(1-2): 98-106.

25. Dileep K, Syed İR. Age-dependent paraoxonase 1 (PON1) activity and LDL oxidation in Wistar rats during their entire lifespan. Scientific World Journal 2014; 1-6.

26. Taha N, El-Noeman S, Korshom M, Mandour A, El-Feky M. (2010) Serum glutathione peroxidase and paraoxonase-1 enzyme activities in camel (Camelus dromedarius): effect of age and sex differences. Journal of Camel Practice and Research 2010; 17: 1-8.

27. Wan-Fen L, Lucio G, Clement E. Serum paraoxonase status: a major factor in determining resistance to organophosphates. Journal of Toxicology Environmental Health 1993; 40: 337-346.

28. Stoltz DA, Ozer EA, Taft PJ, Barry M, Liu L et al. Drosophila are protected from Pseudomonas aeruginosa lethality by transgenic expression of paraoxonase-1. Journal of Clinical Investigation 2008; 118: 3123-3131.