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A New Perspective on Animal Improvement: Metabolomics

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ABSTRACT In order to obtain a high yield from animals, improvement studies were carried out by various methods. Until 1990's, improvement studies were carried out only with phenotypic methods. With the rapid development of genetic science, the possibilities of using genome analysis in improvement studies were investigated. One of the genomic analysis sections is the functional gens. In order to be able to reveal the efficacy of the functional gens, the possibilities of using metabolomics in animal improvement have begun to be investigated. There are nearly 20000 metabolites in living beings. When the relationship of these metabolites with yields, diseases and adaptations of livestock animals is revealed, the use of metabolites in animal improvement is thought to make selection more efficient.

Keywords: *Metabolomic, Selection, Animal improvement*

ÖZ

Hayvan Islahında Yeni Bir Bakış: Metabolomik

Hayvanlardan yüksek düzeyde verim elde edebilmek için çeşitli yöntemlerle ıslah çalışmaları uygulanmıştır. 1990'lı yıllara kadar sadece fenotipik yöntemlerle ıslah çalışmaları yapılmıştır. Genetik biliminde hızlı gelişimle birlikte ıslah çalışmalarında genom analizlerinin kullanılma olanakları araştırılmıştır. Genomik analiz bölümlerinden birisi işlevsel genlerdir. İşlevsel genlerin etkinliğinin ortaya konabilmesi için hayvan ıslahında metabolitlerin kullanılma imkânları araştırılmaya başlanmıştır. Canlılarda 20.000'e yakın metabolit bulunmaktadır. Bu metabolitlerin çiftlik hayvanlarının verimleri, hastalıkları ve adaptasyonlarıyla ilgili ilişkileri ortaya konulduğunda, hayvan ıslahında metabolitlerin kullanılmasının seleksiyonu daha etkin yapacağı düşünülmektedir.

Anahtar Kelimeler: *Metabolomik, Seleksiyon, Hayvan ıslahı*

INTRODUCTION

Though mankind has unlimited expectations from earth, the earth only has limited resources to meet them. When mankind moved into settlements, they also increased the types and amount of production for these limited resources (Ünsal 2013). Yet, as the human population increases rapidly, the need for food material also increases in astounding rates. Mankind tries to meet this food demand with various phytonutrients and animal products (Akçapınar and Özbeyaz 1999).

As there is only a limited amount of space available for agricultural purposes, civilizations now try to improve the animals with hopes to obtain the maximum amount of yield from each animal (Akçapınar and Özbeyaz 1999). In order to be able to increase future yield performances of agriculture and livestock breeding sectors, rapid and accurate estimations have to be made regarding the potential output values for the improvement efforts on the current stock (Aksoy 2003) until the 1990's, the estimations for breeding properties of animals were made based upon their phenotypes and yield parent performances, and genetic improvements were solely based on phenotypic selection (Montaldo and Meza-Herrera 1998). The genetic development of yield characteristics in livestock animals is somewhat slow due to various reasons: some performance values are measurable in only one sex, some yields are based on cumulative effects of multiple genes, and environmental conditions have strong influences on quantitative characteristics. These reasons also lower the accuracy of genetic evaluations. Furthermore, the generation gap is also of a larger size than the optimal due to yield performances being measurable in adult animals only, causing a slower genetic improvement rate (Lara et al. 2002). In recent years, the chance of applying genome analyses are also being investigated in order to obtain a faster genetic improvement rate in yield related characteristics.

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1. GENOMICS

Genomics is the scientific branch which identifies all the genes that code the structural and operational functions of a given organism, and inspects the relationship of these genes with each other and with the environment, along with their production and activation controls in terms of time, amount, and location. Genomics also enters, classifies, and stores the obtained data in computer databases (Yarman et al. 2003). Genome analyses are conducted with structural, functional, and comparative methods. Functional genomics studies on the biological functions of genes, how these are managed, and how they are reflected in the final products (Bal and Budak 2013). Structural genomics focuses on revealing the genetic map of a given organism by genetic and physical mapping and identification of DNA and base strains (Çevik 2005). The revolution in methods for estimating breeding values is in the area of genomics (Blasco 2012).

It is possible to group the application areas of genome analyses on animal improvement and genetics branches in two main categories: short-term (practical) and long-term. The short-term (or practical) applications consist of individual identification, parent identification, controls for genetic diseases, estimation of genetic distances, and determination of offspring sex and twin pregnancy before implantation. Long-term applications, on the other hand, are the creation of genome maps, identification of quantitative trait locus, marker-enhanced selection, and preservation of genetic variation and resources (Gürses and Bayraktar 2014).

2. PROTEOMICS

This science branch tries to illuminate the structures, positions, amounts, post-translation modifications, functions within cells and tissues, and interactions with other proteins and macromolecules for all the proteins given in a specific time and location (Varga 2004). A study on proteomics, for example, has identified the protein components and protein modifications for the *Phanerochaetachrysoporium*, which is a biotechnologically important fungus with the capability to completely mineralize the lignin in the woody plants and destroying the phenolic pollutants. This has led to the exploration of the global expressive genetic profile of the fungus and enabled the scientists to understand the response it gives to metal stress in the molecular level. The alteration in the proteomes of the organism after being subjected to heavy metals was compared to its reference proteome map, and at least 200 types of proteins were found to have altered expressions, illuminating the underlying mechanisms for the microorganism's stress response (Özcan at al. 2007).

3. METABOLOMICS

The "Human Genome Project" was completed in 2003, and it revealed that human body contained approximately 30.000 genes, of which 99.99% were identical in all individuals, while that 0.1% made all the difference (Venter 2003). Understanding this 0.1% difference will be very important in finding out why some people carry the risk for a particular disease, why the prognosis of diseases follows differently in different individuals, or why some people give better reactions to a given medical treatment compared to others. While identifying the genes have not revealed the whole picture for the unknown variables, the roles of these genes became the focus of many new studies, and proteomic and transcriptomic analyses were conducted on them. Yet, even the information revealed by these new studies weren't enough to explain all the phenotypes: this is perhaps because the final form of the information that shapes the phenotype is hidden in the metabolites within a cell (Bren 2005).

Metabolomics is the emerging field of metabolome analysis that identify, quantify, and characterize a large number of metabolites in biological samples (e.g., milk, plasma, and serum), providing interesting insights into the so called intermediate phenotypes that lie in the middle between the genomic space (or level) and the final or external phenotypes, that in livestock might be production traits such as growth rate, milk production, fat deposition, and other economic relevant traits. (Fontanesi 2016).

Metabolites are chemical components that emerge during various reactions within an organism, and are not stored inside the body but instead are transformed into other compounds. Metabolomics is the science branch which works on identifying and measuring the level of small-molecule metabolites emerging from lipids, carbohydrates, vitamins, hormones, and other cellular components inside cells, tissues, and physiologic liquids in a given time period, using highly efficient technologies. Metabolism is a significant factor in determining the phenotype of an organism; thereby the identification of distribution of these metabolites is an important step in the preparation of functional gens. Analyses of various metabolites have been part of the research for biological indicators of health and diseases for many years (Başaran et al. 2010).

Metabolomics is allowing researchers to focus on measuring the end-products of complex, hard-to-decipher genetic, epigenetic and environmental interactions (Goldansaz at al. 2017).

Metabolomics in its close definition is a rather young field in farm animal production. Initially, metabolomic analyses in farm animals had been initiated for many non-genetic applications e.g., control of drug abuse, control of embryo and oocyte quality in reproductive processes or for detection of product origin of food, whereas genetic variability essentially has been ignored in these fields. Only recently, the fields Physiological Genomics/Genetics and Refined phenotypic description of animal models have emerged, that fit into the current concept entitled Genetics meets Metabolomics: from Experiment to Systems Biology. (Kühn 2012).

The genes within a cell relay orders to cell members in order to function properly. Various biochemical reactions take place as a result of these orders, which result in the formation of metabolites. Revealing the relationship between these metabolites and properties like diseases, yields, or adaptations also reveal the actual phenotype regarding that particular property. In a way, genomics and proteomics give information on "what may be", while metabolomics gives information on "what is". As such, detailed and quantitative measurements on metabolites (metabolomics) are the ideal method for identifying the effects of a disease or toxic agents over the phenotype (Coşkun 2007). Biochemical parameters and introductions genes as well as detailed studies on the molecular level are very often used for the recognition of different biomolecules structures of herd (Demir and Mert 2015). Testis, sperm, and its biochemical composition are important for determination of variations between the rams chosen for breeding (Mert at al. 2009). The exact number of metabolites in living organisms are unknown but are thought to be somewhere between 2.000 (minimum) and 20.000 (maximum) (Bren 2005).

Metabolomics is a multi-disciplinary science involving biology, chemistry, and math. It requires utilization of techniques like chromatography results combined through multi-variable data analysis methods, molecular spectroscopy, and mass spectrometry. Two technologies are used at the heart of metabolomics studies: NMR's and various mass spectrophotometry measurements (German et al. 2005).

In order to be able to use metabolites for selective breeding purposes, the factors that cause them to emerge must be identified accurately. These factors are usually species, race, age, yield period, and medical conditions of the animals. Metabolites can be used as bio-indicators for various diseases, and correlations between them and various yield performances, adaptation capabilities, and disease resistance of animals have been researched in order to find ways of using them for selective purposes.

The metabolites used in animal raising:

a) Glutathione: Glutathione consists of glutamic acid, cysteine, and glycine amino-acids, and is present in all cells in ample amounts. It was first discovered by Hopkins in 1921 and later synthesized by Harrington and Mead in 1935 (Bildik et al. 1998). The blood glutathione level is constant in many adult mammals for all the lifetime (Atroshi 1979).

Glutathione plays important roles in intracellular reduction reactions, catalysis events, metabolic functions, and transportation of amino-acids. It protects the cells against free radicals, reactive oxygen types, and toxic compounds of endogenous or exogenous origins (Murray et al. 1993; Rose 1984). Glutathione (GSH) is also a strong anti-oxidant which easily enters into reactions with harmful products resulting from lipid peroxidation and an increase of free radicals, thereby eliminating them (Anderson 1998; Bucak et al. 2010).

GSH takes part in cell metabolism and is an essential compound for the preservation of cell integrity (Kehrer 1993). In addition to directly cleaning out free radicals, it acts enzymatically together with GSH-Px (Glutathione Peroxidase) as well. Furthermore, it also functions as the co-factor of numerous protective enzymes. It helps regeneration of important anti-oxidants like E and C vitamins, returning them to their reactive forms (Valko et al. 2007). Glutathione also has important roles in amino-acid transportation and DNA and protein synthesis within the cells (Tabakoğlu and Durgut 2013).

The connection between GSH levels and milk yield (Rizzi et al. 1998), wool yield (Hoey 1984), wool weight, number of lambs given birth to (Atroshi 1979), and nutritional performance (Yaman et al. 1987) of goats were inspected, and a positive correlation was found between with milk and wool yields, wool weight, number of lambs given birth to. It has been claimed that a selective breeding program for nutritional performance based on GSH levels may lead to increased live weight for goats. Another study has reported that GSH type sheep has increased number of lambs born, and increased birth weight, with improved wool growth rates (Boad et al. 1974). Atroshi and Sandholm (1982) have determined the erythrocyte GSH levels of Fin sheep as a distinct indicator for milk production. Their research reveals increased GSH levels in parallel to increased milk yield. The literature also reports that GSH has influence over milk yield, a number of lambs given birth to, wool growth rate, and wool weight (Atroshi and Sandholm 1982; Boad et al. 1974; Hoey 1984; Rizzi et al. 1998). In a study conducted to determine blood and milk biomarkers for diagnosis of the clinic and sub-clinic mastitis, both milk GSH and serum GSH levels were found to be highest in the control group, followed in order by subclinical mastitis group and mastitis groups, and the differences between the groups were found to be statistically relevant (Sadek at al. 2017).

In another study, Holstein heifers of 216 days age and 210 kg weight were given 2 different doses of HMBI (Methionine, 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester), and the difference between groups in terms of daily live weight and GSH-Px was found to be statistically significant, and increased treatment doses lead to increased GSH-Px values, increasing the overall anti-oxidant capacity (Han et al. 2017). Another study has reported that GSH-Px and GSH could be used as post-partum stress indicators for goats (Manat et al. 2017).

In their study, Çolakoğlu et al. (2017) propose that measurements of GSH-Px levels can be useful in the evaluation of milk production capabilities Holstein dairy cattle. In another study, the same researchers displayed that GSH-Px activity varied between seasons.

It is believed that once the effects of potential environmental influence factors on GSH levels for livestock animals -like species, race, age, season, and sex- are determined, GSH levels can be used as selection criteria for disease resistance, yield properties, and adaptation capabilities.

b) Hyaluronidase: Acrosin, hyaluronidase, esterases and acid hydrolases areacrosomal enzymes (Garner and Hafez 2000). Hyaluronidase is involved in many biological functions like shrinking of cancerous cells, microvascular permeability, allergic reactions, inflammations, defense against certain pathogens and malignities, recuperation of wounds, and fertilization in mammals (Belem-Gonçalves et al. 2006; Krishnapillai et al. 1999; Tanyıldızı and Bozkurt 2002). It is also used in the dissolution of cumulus oophorus matrix (Tanyıldızı and Bozkurt 2003).

Millions of spermatozoids spilled inside the genital organ of the female through artificial insemination or natural copulation use their tail movements and the natural suction occurring during the copulation to move inside the uterus, and then towards the final parts of the oviducts. Once here, they undergo a physiological alteration period which increases their movement and proteolytic enzyme secretion capabilities, which are necessary to inseminate the eggs in the infundibulum (or the initial 1/3 portion of the oviduct). This phenomenon is called "capacitation". Once inside the oviduct, capacitation takes 5-6 hours in cows, and 1-1.5 hours for sheep. In order for the egg to be inseminated, the membrane made of granulosa cells surrounding the egg has to be dismantled and the egg has to be set free. This is only possible with the hyaluronidase enzyme secreted by the spermatozoids. Since the enzyme secreted by a single spermatozoid is not enough, the egg has to be surrounded by many of them. Once they are of enough numbers, one of them can penetrate the egg membrane with its head, while the tail dismantles and is left outside. The head of the spermatozoid merges with the egg nucleus, completing the fertilization. The researches conducted indicate that low sperm hyaluronidase activity may lead to reduced fertilization capability of sperms (Tanyıldızı and Bozkurt 2002; Tanyıldızı and Bozkurt 2003).

Reddy et al. (1980) have used a hyaluronidase inhibitor in rats to clarify the functions of HYAL (Arslan et al. 2016). In their study, it was revealed that miyoclizyne, a natural HYAL inhibitor that doesn't affect the acrosome reaction,

could prevent insemination due to reduced COC (cumulus-oocyte complex) dissemination.

In another study made to reveal the HYAL enzyme activity in various sheep species, Kıvırcık rams were found to have the highest enzyme activity. The researchers claim that these results were inspected for fertilization yield, and they could be used for pre-characterization studies for marker-enhanced selection (Tiryaki and Temur 2010).

c) PON1 (Paraoxonase 1) Enzyme: A major stress source for the animals are the pesticides they consume either directly or through their feed. Once applied to a field, 0.015-6% of the pesticide effects the targeted organism, while the remaining 94%+ reaches to the environment, soil, and organisms that were not targeted at all (Tiryaki and Temur 2010). The effects of pesticides on living organisms can be inspected under two categories, as acute and chronic effects. Acute effects vary from mild allergic reactions to death. Chronic effects, on the other hand, consist of neurotoxicity, behavior abnormalities, lowered reproduction and fertility rate, and mutagenic, teratogenic, and carcinogenic effects (Tarakçı and Türel 2009). Paraoxone is a catabolic product of parathion compounds used in insecticides to obtain increased agricultural yields. It inhibits the acetylcholine esterase enzyme used in nerve impulse transmittance, along with some other enzymes. The organisms, on the other hand, have developed some defense mechanisms against most of these effects. One of these mechanisms is the paraoxonase (PON) enzyme. This enzyme hydrolyzes the paraoxone, preventing its harmful effects (Costa et al. 1999). This activity reveals the detoxification effects of the enzyme and is important against cancer caused by environmental factors. Furthermore, paraoxonase also has anti-oxidant activity as well.

PON1 enzyme prevents the damage caused by toxins originating from organophosphate compounds. It also has protection role against plasma LDL's lipid oxidation, and some bacterial endotoxins (La et al. 1999). As mentioned before, organophosphates affect the organism by inhibiting the acetylcholine esterase (AChE). AChE is an enzyme which hydrolyzes the enzymes and some neuro-transmitters in somatic and parasympathetic nerve systems. Paraoxonase is a potent inhibitor for the choline esterase which destroys acetylcholine. This causes repeated neuron impulses and acetylcholine accumulation in synaptic junctions. In mammals, even if an oxoneorganophosphate escapes detoxification in the liver, it may still get hydrolyzed by PON1 enzyme inside the serum, before it reaches the target area. Inhibition of this enzyme may cause poisoning with organophosphate agents (OP), and some other malfunctions in the nervous system (Vera et al. 1998).

Paraoxonase enzyme also hydrolyzes organophosphate-based nerve gases like soman, sarin, and tabun, transforming them into less harmful compounds (Hughes et al., 2002). The enzyme also hydrolyzes the toxic oxone metabolites of various insecticides like parathion, diazinon, and chlorpyrifos (La 2002).

Since it's an anti-oxidant enzyme it is believed that paraoxonase has protective roles against cardiovascular diseases, diabetes, sepsis, and Alzheimer's, and Parkinson's diseases (Draganov and La 2004; La 2002).

Cattle of various races have displayed reduced amounts of standard PON1 levels during the peak period of the lactation (Kulka et al. 2016). In one study, blood samples were collected from dairy heifers of varying ages in transition periods in order to analyze for beta-hydroxybutyrate (BHB), free fatty acids (FFA), PON1, and total antioxidant capacity (TOC) to determine the oxidative stress and other metabolic disorders. The results of the study indicate the presence of positive correlations between HDL-C, total antioxidant capacity (TOC), and PON1 activity. It is also revealed that PON1 activity changes along with the lipid metabolism and negative energy balance (Turka et al. 2013).

Oxidative stress harms over follicles, oocytes, and normal metabolic events. Plasma PON1 activity in the Holstein cattle in early lactation period was found to be twice that of PON1 activity in their follicular liquid (Schneider 2013). PON1 is present in various levels in various tissues, not only in humans but also in animals. Paraoxonase has been obtained from rabbits, rats, sheep, cattle, fish, and mice, and various studies have inspected its structural properties (Arslan et al. 2011; Clement et al. 2002; Erol et al. 2013; Erzengin et al. 2014; Furlong et al. 1991; Vera et al. 1998). It has also been revealed that race, age, lactation period, and health were factors that influenced PON1 enzyme activity levels in cattle (Antonic-Svetina et al. 2011; Erzengin et al. 2014; Giordino et al. 2013).

Furthermore, in a study conducted on Drosophila melanogaster which is devoid of PON enzymes, transgenic flies were obtained first, after which the effects on the virulence of Pseudomonas aureginosa were investigated. It has been revealed that the obtained transgenic PON1 protected the flies against Pseudomonas aeruginosa's deadly effect, and created resistance in the flies against organophosphates (Stoltz et al. 2008).

d) Adenosine Deaminase (ADA): ADA is a polymorphic enzyme present in all the cells of the body, and it plays an active role in the metabolism of the nucleotides. It catalyzes the irreversible and hydrolytic deamination of deoxyadenosine to deoxyinosine, which is a reaction step for the purine metabolism. The enzyme is present in all the cells, and its activity can be measured in the blood, tissue and body fluid samples. That being said, it has a higher presence in the spleen, lymphocytes, and erythrocytes (Ungerer et al. 1992). The enzyme has to alleles named ADA1 and ADA2, and ADA2 activity was found to be 35% lower compared to ADA1's (Battistuzzi et al. 1981).

It is also reported that infertile women, and babies with lower birth weight, have lower ADA2 enzyme activity as well (Bottini et al. 1981). A positive correlation was found between birth weight and placental weight in the individuals with ADA 2 allele gene ADA 2 (Gloria-Bottini et al. 2008). Napolioni (2010) claims that adenosine levels are not only required for oocyte maturation but are also critical for intra and extrauterineselection. The enzyme is also known to have a special role in cellular growth and cell differentiation (Hershfield and Mitchell 1995). ADA activity hence becomes an important target during chemotherapy applications. ADA deficiency has been associated with many important problems, particularly with immune deficiency (Bahadır 2009).

Interestingly, this enzyme also takes part in post-mortem nucleotide metabolism and contributes to the taste of the meat by destroying the nucleotides, causing the formation of imozyneandy hypoxanthine (Alrokayan 2002). The relationship between this enzyme –which has been associated with various diseases, birth rate, and meat quality- should also be researched for its other properties, and whether these properties may be of use for selection of animal groups for breeding.

When the studies on metabolites are inspected, it can be seen that they are mostly conducted by biochemistry,

biology, and veterinary clinical sciences academicians. The studies that were conducted by biochemistry and medical sciences were found to have been limited to species and age properties, and studies regarding the race property – which has an influence on the metabolites- were non-existent.

As a result, considering there may be up to 20.000 types of metabolites in the farm animals, and from the perspective of the above information, it is believed that researching the subject in terms of the races within the same species, and searching for the ways of utilizing the metabolites in selection programs, would contribute greatly to the animal improvement efforts, and obtaining increasing yields.

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