NON-INVASIVE TECHNIQUES FOR EXACT PHENOTYPIC ASSESSMENT OF CARCASS COMPOSITION AND TISSUE GROWTH IN DOMESTIC ANIMALS [1]

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ABSTRACT

The present review paper deals with the advances in exact phenotyping of domestic animals regarding carcass composition and the changes in tissue proportions during growth. The development of non-invasive methods such as computer tomography (CT), dual energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) is presented, especially in relation to recent investigations of genetic aspects of growth. Supported by various bio-statistical approaches that can manage huge amount of data, such research could enable better understanding of complex phenotypic traits like changes in body composition during growth.

Key words: domestic animals, phenotyping, non-invasive methods, carcass composition, growth

1 INTRODUCTION

In early stages of animal body composition studies, only destructive methods were at disposal for scientists. The methods commonly used at those times were total dissection, followed by separation into major tissues (Davies and Kallweit, 1979; Vincek *et al.*, 2010). Other methods, as well, can be found in literature such as grinding followed by chemical analysis (Tess *et al.*, 1986; White *et al.*, 1995).

During the last 30 years, however, a number of non-destructive or non-invasive methods evolved which can accurately depict body composition of domestic animals, most widely used being ultrasound, computer aided tomography (CT), dual energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) (Scholz *et al.*, 2015).

CT has been used to measure body composition in farm animals for quite some time, for example in sheep breeding (Young *et al.*, 2002; Bunger *et al.*, 2011), rabbit (Szendro *et al.*, 2012), beef (Navajas *et al.*, 2010) or pig

body composition analyses (Font i Furnols *et al.*, 2009; Kongsro, 2014). Recently the introduction of CT as a reference method for pig carcass classification was proposed (Daumas and Monziols, 2011). Similarly, DXA can be used in the evaluation of the body composition of pigs both *in vivo* and on carcasses of with a high accuracy (Scholz and Förster, 2006; Scholz *et al.*, 2006, 2015; Bernau *et al.*, 2015).

To some extent better results when compared to DXA in the investigation of body composition demonstrated the usage of MRI in pigs by Bernau *et al.* (2015) and Lee and Kuk (2013) in humans. In the latter case, multiple image protocols that cover the entire body are used as the reference method for the whole-body composition assessment.

There are a number of studies which showed that body composition of the pig can be accurately predicted by an acquisition of a series of cross-sectional images covering the whole carcass or primal cuts (Mitchell *et al.*, 2001; Tholen *et al.*, 2003; Baulain *et al.*, 2004; Collewet *et al.*, 2005; Monziols *et al.*, 2006; Olsen *et al.*, 2013;

Kremer *et al.*, 2013). The major advantage of using the non-invasive techniques is the ability to perform investigations on living animals without detrimental consequences to the animal. For that reason, these methods are suitable when sequential studies of the same object are required. This is particularly desired when conducting longitudinal studies of phenomena such as growth, development, ageing, and seasonal influences or environmental factors.

Based on repeated measures of cross-sectional MR images on the same animals, tissue composition of growing pigs and their body parts were examined in studies of Kastelic *et al.* (1995), Baulain *et al.* (1996) and Kusec *et al.* (2007). DXA allows an even easier approach, because whole body composition results are available immediately after scanning as was demonstrated in DXA growth studies on calves e.g. by Scholz *et al.* (2003) or in pigs by Scholz and Mitchell (2003) and Mitchell and Scholz (2007, 2008).

## 2 PHENOTYPIC EVALUATIONS OF ANIMALS' GROWTH IN LIVE WEIGHT AS WELL AS IN SPECIFIC TISSUES

Growth can be considered as complex phenotypic trait that results from a number of biological processes in the animal. According to Schinkel and Einstein (1995), the genotype determines the maximum level at which these processes can occur, whereas environmental factors determine the degree to which genetic potential is expressed. Thus, an understanding of the relationships between genotype and environmental factors is essential in order to implement strategies that could improve utilization of growth potential. With this in mind, Kusec

et al. (2007) investigated growth characteristics of hybrid pigs distributed in four groups according to genotype at malignant hyperthermia syndrome (MHS) gene (NN and Nn) and feeding system (*ad libitum* and restrictive). The analysis of growth by asymmetric S-function showed different patterns of live weight growth of pigs from two feeding regimes as shown on the Figure 1a.

Within the feeding regimes, no significant differences in live weight growth patterns between the pigs of different MHS genotype (NN and Nn) were found.

Muscle growth pattern determined by 4 consecutive MRI measurements at 4 week interval significantly differed between the groups of investigated pigs (Fig. 1b); Nn pigs from the intensive feeding group were significantly superior to NN pigs in the same group and to Nn pigs fed restrictively ($p < 0.05$), but only in the phase of progressive growth (b-coefficient of the S-function). In this respect Nn pigs performed better under intensive feeding than under restrictive feeding regime, while no difference was found between NN pigs from two feeding regimes. This led to the conclusion that cost effective restrictive feeding regime can be recommended as more appropriate in fattening of pigs, since intensive feeding generally failed to improve their muscle growth. Similarly, authors found that inclusion of MHS-gene did not enhance muscle growth characteristics of investigated pigs, so MHS-negative pigs (NN) can be considered as more desirable fatteners, especially when fed restrictively.

Furthermore, on the basis of MRI measurements of muscle and live weight analyses by generalized logistic S-function, Kušec *et al.* (2008) found that the optimal slaughter time/weight for pigs in terms of maximum muscle growth potential. In their study, this point was described as the time when the intensive phase of muscle growth ceased and turned to the phase of retardation
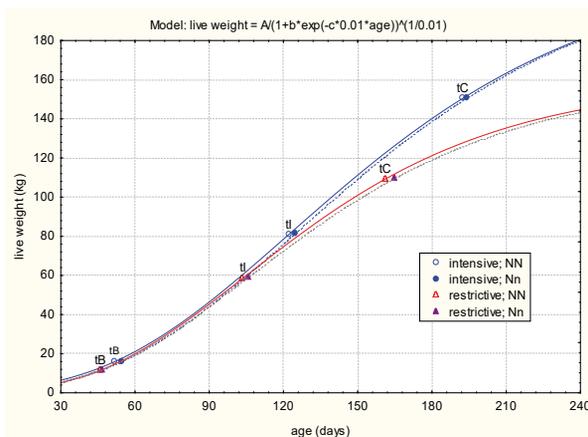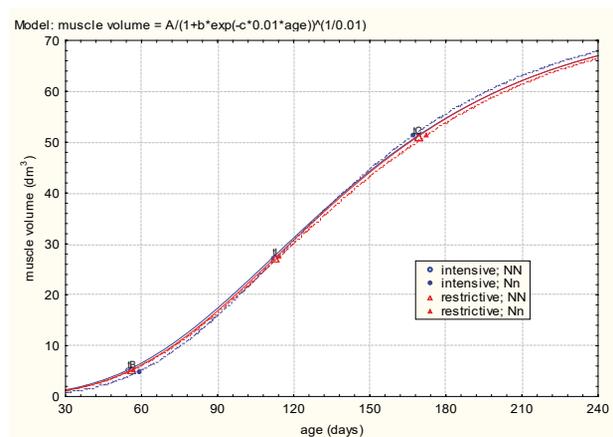
(a)   (b)

Figure 1: (a) Live weight growth curves of two MHS-genotypes (NN and Nn) of pigs kept on intensive and restrictive feeding regime; (b) Muscle growth curves of two MHS-genotypes (NN and Nn) of pigs kept on intensive and restrictive feeding regime

Table 1: Deposition rates for protein, fat and bone mineral (g/d) in pigs with different Ryanodine Receptor 1 (RyR1)-Genotypes [LSM ± SEE; modified from Scholz and Mitchell, 2003]

Growth Period (live weight, kg)	RyR1-Genotype								
	NN			Nn			nn		
	Deposition rate (g/d)								
	Protein	Fat	Bone Mineral	Protein	Fat	Bone Mineral	Protein	Fat	Bone Mineral
1.2–10	42 ± 3	13 ± 2	6 ± 1	39 ± 4	10 ± 2	5 ± 1	30 ± 4	8 ± 3	4 ± 1
10–30	104 ± 4	57 ± 4	13 ± 1	107 ± 3	59 ± 3	13 ± 0	115 ± 5	58 ± 5	14 ± 1
30–60	**120 ± 6**	199 ± 13	**19 ± 1**	**123 ± 5**	**172 ± 11**	**19 ± 1**	**132 ± 7**	**168 ± 16**	**19 ± 1**
60–90	104 ± 14	225 ± 45	9 ± 1	**116 ± 14**	183 ± 45	9 ± 1	112 ± 19	125 ± 58	12 ± 1

which occurred when muscle volume reached of 51.31 dm³. For pigs from the intensive and restrictive group, this moment occurred at the age of 164 and 167 days when their weight was estimated to 126 and 112 kg, respectively. After these moments pigs tend to gain weight only by fat deposition.

Slightly different results were found by Scholz and Mitchell (2003) using DXA. Protein (muscle) deposition rates found their maximum already between 30 and 60 kg live weight showing lower protein deposition rates between 60 and 90 kg live weight, while fat deposition rates were already higher than lean deposition rates between 30 and 60 kg live weight (Table 1).

NN and Nn did not differ significantly during growth in both cases (Kusec *et al.*, 2008; Scholz and Mitchell, 2003), while nn (not studied by Kusec *et al.*, 2008) showed a significantly higher protein deposition rate between 30 and 60 kg body weight.

3   GENETIC/GENOMIC STUDIES OF BODY COMPOSITION DURING GROWTH

In the meantime genetic analyses evolved to a great extent. Genome research in domestic animals is generally conducted in the aim to be applied in livestock production practices. The achievements made in that directions were most certainly a great stimulus for genome sequencing programs in domestic animals while molecular information became very important in practical breeding programs. Silva *et al.* (2013) studied genomic growth curves of an outbred pig population. The authors postulated that the genetic basis of pig growth can be understood by assessing SNP marker effects and genomic breeding values based on estimates of these growth curve parameters as phenotypes. They compared two statistical approaches using empirical weight-age data from an outbred F2 (Brazilian Piau X commercial) population. On the basis of the obtained results they constructed genomic growth

curves, which showed substantial genetic discrimination among animals in the final growth phase. The SNP effect estimates allowed identification of the most relevant markers for each phenotype, the positions of which were coincident with reported QTL regions for growth traits. Andersson (2015) presented a genetic dissection of major locus responsible for the control of muscle growth and fat deposition. In this review he described the identification of single-nucleotide substitution in intron 3 of the gene for insulin-like growth factor 2 (*IGF2*) that is underlying a quantitative trait locus affecting muscle growth, size of the heart, and fat deposition. As it is obvious, advances in genetics and genomics with their cutting-edge biochemical methods and subtle statistical approaches make powerful tools in the explanation of complex biological phenomena such as animal growth.

Inclusion of non-invasive techniques with their ability to give insight in the changing body composition of animals during growth can help to broaden the knowledge on these phenotypes. Since proportions of tissues in the carcass of domestic animals, like muscle and fat, influence the value of the final product, that is primarily processed carcass, the traits considered here are also of economic importance. That is why we conclude that non-invasive methods should be used in the phenotyping of meat animals for the growth traits in more detail than live weight – also in order to decrease the mismatch between wide available molecular data and phenotypic data that are often based on less reliable slaughterhouse standards only (see also Bruford *et al.*, 2015). This would in turn improve the animal breeding programs aimed at more effective meat production by the use of genetic analysis of animal's growth in live weight, muscle, fat, and not to forget bone tissues.

First reports, where non-invasive techniques in farm animals were combined with genome wide association studies (GWAS), originate from the pig as model animal (Kogelmann *et al.*, 2013) or as farm animal (Rothammer *et al.*, 2014). DXA as a simple and affordable
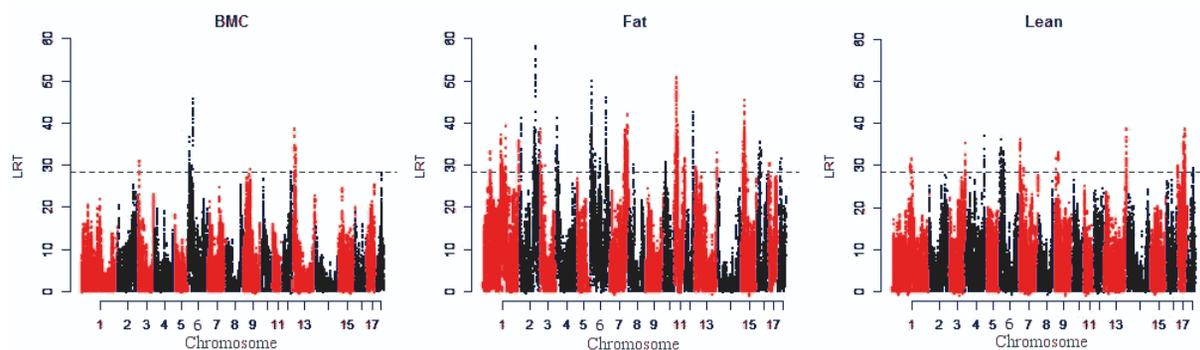
Figure 2: Manhattan plot of GWAS results for DXA phenotypes (BMC = bone mineral content, Fat, and Lean) in pigs (modified after Rothammer et al., 2014)

non-invasive technique has been used in both cases to providing the body composition and bone mineralization phenotypes for the genome wide association study. Rothammer *et al.* (2014) identified a large number of with body composition associated SNP's, which were not known before to be associated with body composition or bone mineralization (Fig. 2).

Furthermore, Matika *et al.* (2016) recently performed a genome wide association study about the growth and carcass traits of lambs based on computed tomography (CT) measurements on live animals. They identified quantitative trait loci (QTL) on OAR1, 3, 24 and OAR6 that are associated with the traits of major tissues (muscle, fat and bone). Authors stressed that revealed single nucleotide polymorphisms (SNPs) have a potential for selection on improved carcass composition and meat quality in sheep breeding. Further investigations are recommended in order to clarify if the effects on QTL OAR6 are caused by a single gene or more closely-linked genes. This work represents the fine example of exploiting the improved genotyping tools (the Illumina OvineSNP50 SNP chip) by which up to 54,241 independent SNPs can be simultaneously characterised, in combination with non-invasive measurements of carcass composition by CT in order to identify QTL and candidate genes with significant effects.

Nowadays, the genetic gain on body composition of breeding pigs is extensively studied using CT measurements. For example, as a part of Norsvin (now Topigs-Norsvin) breeding system, 3500 boars are scanned annually in Norway (Kongsro, 2014). An estimated 30 % increase of genetic gain on lean meat percentage by the use of this non-invasive technology for collecting the data for phenotyping *in vivo* has been reported.

Due to such promising results as those presented in this review, it is to be expected that similar research will be performed more frequently in near future giving insights in genetics behind such complex traits as growth of animals and humans as well.

4   CONCLUSION

Growth and development of whole animals as well as the growth of tissues, single muscles and organs is getting a new interest in animal science. It is becoming to be viewed as a complex phenotypic trait with great importance as in fact the basis of animal production.

Due to its ability to perform investigations on living animals without detrimental consequences to the animal, non-invasive techniques such as ultrasound, computer aided tomography (CT), dual energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) are becoming increasingly appealing to the animal scientists, especially those interested in the genetic aspects of growth.

It seems that a growing interest emerges in combining the non-invasive techniques for body composition studies with up-to-date genetic and genomic analyses. Supported by various bio-statistical approaches that can manage huge amount of data, such combination represents a promising way to the better understanding of such complex phenotypic traits as body composition during growth, an ever changing morphological set up of domestic or other animals; and humans.

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