World Rabbit Science

THE GENE EXPRESSION OF WEANING AGE AND ITS EFFECT ON PRODUCTIVE PERFORMANCE OF RABBITS

EL-SABROUT K.*, AGGAG S.A.†

*Department of Poultry Production, Faculty of Agriculture, Aflaton St., El-Shatby, PO Box 21545, University of Alexandria, ALEXANDRIA, Egypt.
†Department of Genetics, Faculty of Agriculture, Aflaton St., El-Shatby, PO Box 21545, University of Alexandria, ALEXANDRIA, Egypt.

Abstract: Weaning age for mammals remains a topic of debate and an interesting subject of research. The literature data reflect opposite views on the recommended weaning age of rabbits. Thus, we determined the optimal weaning age for average commercial rabbit lines by studying one of these lines, the V-line. Gene expression of weaning age was studied in this research to reach the optimal weaning age for efficient rabbit growth and survival. The effect of weaning age on growth and mortality rates was investigated in young rabbits by comparing 3 groups (kits of 10 V-line does for each group), weaned at 23 (W23), 28 (W28) and 33 (W33) days of age. Rabbits weaned at 23 d of age had significantly ($P<0.05$) lower body weight at the age of 63 d (market age) than those weaned at 28 and 33 d of age. The weaning age also influenced survival; mortality rate was highest in rabbits weaned at 23 d of age, followed by those weaned at 28 and 33 d of age. Morphometric parameters reflect the integral effect of all factors influencing digestive tract growth and development. From the results, it seems that the small intestine length did not have a clear effect on different weaning ages. Quantitative real-time polymerase chain reaction analysis is an important tool to monitor changes in gene expression in animals such as rabbits. We used this approach to measure intestinal insulin-like growth factor-1 (IGF-1) mRNA level and observed that the expression levels of IGF-1Ea, IGF-1Eb and IGF-1R were nearly the same in W28 and W33 rabbits, while they were the lowest in W23 rabbits. Serum IGF-1 concentrations tended to present significant differences ($P<0.05$) with different weaning ages. We found that levels of IGF-1 in rabbits weaned at 28 and 33 d of age were convergent and higher than the IGF-1 levels in rabbits weaned at 23 d of age. Moreover, the early weaning of rabbits has a negative impact on growth. This therefore suggests that moderate weaning (28W) will be suitable for the farm economy and will improve rabbit production better than early or late weaning.

Key Words: gene expression, optimal weaning age, intestine, RT-PCR, IGF-1, rabbit.

INTRODUCTION

As small mammals, domestic rabbits (*Oryctolagus cuniculus*) have many advantages that make intensive production to solve part of the food shortage problem around the world possible. V-line is a synthetic maternal line originated in 1982 at the Department of Animal Science of the Universidad Politecnica de Valencia, Spain (Estany *et al.,* 1989), which was imported to Alexandria University, Egypt. Hoon *et al.* (2010) stated that weaning is a critical period for young rabbits due to separation from the doe (mother), milk withdrawal and adaptation to a new solid feed. The growth and development of rabbits as well as their survival depend greatly on the genotype and management practices adopted during the production cycle. Growth and mortality have a serious economic impact on rabbit meat production, which is affected by the weaning age.

Information on the optimum weaning age for rabbits is contradictory, although weaning is usually carried out between 21 and 35 d of age. Xiccato *et al.* (2003) noted that weaning age affected growth performance. In contrast, Tůmová *et al.* (2006) did not prove any effect of weaning age on body weight of rabbits. Zita *et al.* (2007) found that early weaned rabbits (21 d) had higher body weight than later weaned rabbits. However, Trocino *et al.* (2001) observed that early weaned kits showed a lower body weight in comparison with rabbits weaned later (32 d).

Quantitative real-time polymerase chain reaction (qRT-PCR) is an important tool to monitor changes in gene expression in animals such as rabbits and a highly sensitive technique for the detection and quantification of an amplified PCR product (mRNA) based on incorporation of a fluorescent reporter dye (Logan, 2009).

On the other hand, insulin-like growth factor-1 (IGF-1) is a protein which has a similar structure to insulin and plays a major role in metabolism, proliferation and differentiation in animals including rabbit (Duclos, 2005). It is produced primarily by the liver as an endocrine hormone as well as in target tissues. IGF-1 gene is involved in growth of different tissues, such as muscle (Tirapegui, 1999). Moreover, production is stimulated by growth hormone (GH) and can be retarded by under-nutrition and growth hormone insensitivity.

The purpose of this study was to evaluate the gene expression of different weaning ages (at 23th, 28th and 33th day of age) and its effect on productive performance in V-line rabbits, to determine the optimum weaning age for efficient growth and survival of rabbits.

MATERIALS AND METHODS

The V line rabbits used were from the stock available at the Poultry Research Centre rabbitry, Alexandria University, Egypt, in season 2015/2016. Some 360 rabbits (kits of 30 does) of V-line (mixed sex) were used during the experimental period. Does' litters were randomly divided into 3 groups (120 rabbits per group) of 23 (W23), 28 (W28) and 33 (W33) days of age according to weaning age. The 3 weaning ages were adopted as treatments. Rabbits in this study were housed in cages and given *ad libitum* access to commercial pelleted diet containing 18% crude protein, 13% crude fibre and 2600 kcal/kg (Table 1) with fresh clean water. Young rabbits were moved to other cages at weaning. By using high standard hygiene and good management, the occurrence of dangerous diseases was largely avoided throughout the experimental period.

Effect of weaning age on performance

Data were collected on individual body weight (g) at different weaning ages (23, 28 and 33 d) (BW1), individual body weight (g) at 63 d of age (market age) (BW2), and litter mortality rate (%) from 23 to 63 d of age.

Experimental design and statistical analysis

The experiment was in a completely randomised design (CRD) with age at weaning as factor of interest. Data were statistically analysed using SPSS statistical program (2011). The statistical model was:

$$Y_{ij}=\mu+T_i+E_{ij}$$

Where, Y_{ij}: the observed value of the dependent variable, μ: the overall mean, T_i: mean effect of the ith age at weaning (i=1–3), E_{ij}: the random error.

Table 1: Ingredients of experimental diet and their gross composition for V-line rabbits.

Ingredients	Gross composition (%)
Claver hay	17.1
Barley	33.0
Corn	25.0
Soybean meal (44% crude protein)	20.0
Molasses	3.0
Limestone	1.0
Sodium chloride	0.3
Vitamins & Minerals (premix)	0.5
Coccidostat	0.1

Intestine measurements

1. Intestinal morphological analysis

Slaughtering of rabbits (at 63 d of age) was carried out 3 h after feed removal. Fifteen small intestinal samples (5 samples for each group) were taken and measured

by meter. Data were statistically analysed using SPSS statistical program (2011).

2. The Quantitative Real Time-PCR analysis

Fifteen small intestinal tissue samples (5 samples for each group) (approximately 1 cm in length) (added to RNA Shield™) were briefly rinsed in ice-cold distilled water and immediately frozen in liquid nitrogen. They were crushed until a fine powder was obtained using a mortar (previously sterilised). Total RNA was extracted from samples by a single-step isolation procedure using Direct-zol™ RNA kit supplied with TRI Reagent® (Zymo Research, USA) following the manufacturer's protocol and stored at –80°C until used.

The RNA extracted from intestinal tissues was used as template to examine the expression level of IGF-1Ea, IGF-1Eb and IGF-1R, designed according to published IGF- gene sequence from the literature (Feng and Von Barthend, 2011) (Table 2) in the presence of reference gene Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for rabbit. The results were expressed as a ratio of the target to standard signal.

Table 2: List of growth messengers and the reference gene used in Real Time- PCR for V-line rabbits.

Code	Nucleotide sequence (5'-3')
IGF-1Ea	Forward 5'-AGGAGGCTGGAGATGTACTG-3' Reverse 5'-AAATGTACTTCCTTCTGAGTCT-3'
IGF-1Eb	Forward 5'-CATGCCCAAGACTCAGAAGT-3' Reverse 5'-AAATGTACTTCCTTTCCTTCTC-3'
IGF-1 R	Forward 5'-ACCGCAACTACCGCTTCCCC-3' Reverse 5'-CCGCGGATGACCGTGAGGTT-3'
GAPDH	Forward 5'-ATTGCCCTCAATGACCACTTTG-3' Reverse 5'-TCTTACTCCTTGGAGGCCATGT-3'

Real-time PCR was performed on Stratagene Mx3000P system (Stratagene, USA); reactions were run in duplicate with one-step SYBR Green PCR Master Mix (BIOLINE, USA) according to the manufacturer's default protocol. The real-time PCR program was performed as follows: initial denaturation at 95°C for 5 min; 40 cycles of 95°C for 15 s, annealing at 60°C for 30 s and extension at 72°C for 30 s. Data acquisition was performed during the extension step. For gene expression quantification, comparative Ct method was used. The best primer pairs (lowest Ct, highest amplification efficiency and stable performance) were selected for real-time PCR. The optimal primer concentrations were determined according to the Stratagene real-time PCR protocol. The mRNA levels of the target messengers were calculated by normalising the mRNA levels with that of GAPDH (control). Comparative quantification was statistically analysed according to Livak and Schmittgen (2001).

Evaluation of Insulin-like Growth Factor 1 in blood

Serum was obtained by centrifugation of the blood samples at 4000 rpm for 10 min at 4-6°C and was assayed immediately. Serum Insulin-like Growth Factor 1 (IGF-1) samples were analysed using IGF-1 ELISA kit (SIGMA-ALDRICH®) and the colour change was measured spectrophotometrically at a wavelength of 450 nm±10 nm. The concentration of IGF-1 in the sample was then determined by comparing the O.D. of the sample to the standard curve.

Table 3: Effect of different weaning age (23, 28 and 33 d) on individual body weight at weaning, individual body weight at 63 d, mortality rate from 23 to 63 d and length of small intestine at 23, 28 and 33 d of age of V-line rabbits.

Parameters	Weaning ages			P-value
	W23	W28	W33	
BW 1 (g)	402.73±26.13[a]	613.02±8.61[b]	868.72±13.05[c]	0.045
BW 2 (g)	1210.33±28.07[a]	1467.03±25.03[b]	1543.16±21.22[b]	0.048
M (%)	3.9[b]	1.3[a]	1.1[a]	0.033
Length of small intestine (cm)	226±0.13	229±0.18	231±0.20	0.068

[ab] Means in the same row with different superscripts are significantly different (P<0.05).
W23: weaning at 23 d; W28: weaning at 28 d; W33: weaning at 33 d. BW 1: individual body weight at weaning; BW 2: individual body weight at 63 d. M: mortality rate (%).

RESULTS

Effect of weaning age on performance

Most performance results in this experiment were significantly affected by the weaning age (Table 3). Indeed, the rabbits weaned at 23 d had significantly (P<0.05) lower body weight at the age of weaning compared with those weaned at 28 and 33 d of age. Rabbits weaned at 23 d of age had significantly (P<0.05) lower body weight at the age of 63 d (slaughter or market age) than those weaned at 28 and 33 d of age. The weaning age also influenced survival; mortality rate was highest in rabbits weaned at 23 d of age, followed by those weaned at 28 and those at 33 d of age. Similar results were reported by Giddene and Fortun-Lamothe (2004), who found a higher mortality rate in early weaned rabbits, which indicates that early weaning is not preferable in rabbit breeding.

Intestine measurements

1. Intestinal morphological analysis

The morphometric traits of small intestine, especially the length, are important to understand expression incident as a result of different weaning ages and its impact on digestive potential and then growth.

Our results showed that small intestines have lengthened in rabbits weaned at 28 and 33 d comparing with rabbits weaned at 23 d, without significance (*P*<0.05) (Table 3).

2. The Quantitative Real Time-PCR analysis

The main goal of this analysis was to detect and quantify 3 different specific messenger (IGF-1Ea, IGF-1Eb and IGF-1R) mRNA levels in developing small intestine from rabbits among the 3 groups (W23, W28 and W33), to better understand the role of these growth factors in this tissue.

The present data demonstrate significant differences in gene expression of IGF-1Ea, IGF-1Eb and IGF-1R mRNA levels in the developing small intestine of weaning rabbits. Figure 1 shows a picture resulting from qRT- PCR used for the quantification of IGF-1 mRNA in the small intestine of rabbits weaned at different ages. The amount of total RNA from each intestinal sample served for individual evaluation of all IGF-1Ea, IGF-1Eb and IGF-1R mRNA levels. The intestinal IGF-1Ea, IGF-1Eb and IGF-1R mRNA levels were significantly different. The expression of IGF-1Ea in weaned rabbit's intestine was the highest. However, RT-PCR analysis of small intestine tissues revealed that IGF-1Ea, IGF-1Eb and IGF-1R expression levels of W28 rabbit were nearly the same in W33 while the IGF-1Ea, IGF-1Eb and IGF-1R mRNA messengers in W23 rabbits showed the lowest expression.
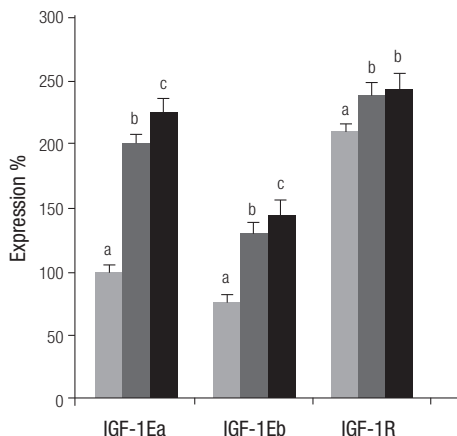

Figure 1: Real Time PCR using 3 growth messengers showed different expression levels with different weaning ages in small intestine tissue of rabbits. ▨ W23, ▨ W28, ■ W33. Means at each growth messenger showing different letters were significantly different at P<0.001.

Evaluation of Insulin-like Growth Factor 1 in blood

Serum Insulin-like Growth Factor 1 (IGF-1) concentrations tended to have significant differences (*P*<0.05) at different weaning ages. We found that levels of IGF-1 in rabbits weaned at 28 and 33 d of age were convergent and higher than the level of IGF-1 in rabbits weaned at 23 d of age (Figure 2).

DISCUSSION

Weaning age is one of several important environmental factors that have an effect on rabbit performance. It is

a management practice that influences the animal's growth, production and reproduction. Weaning traits such as weights and survival/mortality rates are not affected greatly by additive gene action and thus can be improved by good management decisions, among which is the age at which rabbits are weaned.

Knowing the optimum weaning age for rabbits is very important in breeding. The information on optimal weaning age of rabbits is contradictory. From this study, we determined the optimum weaning age for average commercial rabbit lines by studying one of these lines, namely V-line.

Figure 2: Serum Insulin-like Growth Factor 1 (IGF-1) concentrations at different weaning ages of rabbits.

Effect of weaning age on performance

Regardless of the numerous studies, the reported effects of weaning age on growth and development of rabbits are conflicting. Studies relating the age of weaning to growth performance and survival of kits are very scarce in the literature. Hoon *et al.* (2010) stated that weaning is normally a stressful period in the life of young animals, often characterised by a decrease in growth, body weight and increase in mortality rate in some cases. However, they pointed out that weaning shock depends on the age and body weight of young animals. From the above statement, it is obviously true that weaning age affects not only growth performance but also the survival rate of rabbits. Therefore, streamlined research is necessary to help determine the optimal age at which rabbit genotypes could be weaned for increased productivity.

Individual body weight at 23 d for the 3 groups was not significant at all, being due to the high homogeneity of V- line rabbits, and it was not clearly significant enough at 33 d for all weaning age effects to appear

The significant higher post-weaning body weight observed in this study for the W28 and W33 kits and lower mortality rate encourages weaning at 28-33 d for optimal production efficiency without affecting doe health or causing undue stress. These results are in agreement with Fonteh *et al.* (2005), who reported that weaning age influenced growth performance and survival of animal; and Feugier *et al.* (2006) who found that early weaning of rabbits had a negative impact on body fat depots of does, especially in primiparous does.

Moreover, early weaned rabbits (W23) in this study presented lower body weight and higher mortality rate than other weaned rabbits. These results are also in agreement with some previous studies (Gallois *et al.,* 2004 and Bivolarski *et al.,* 2011). Besides, the early withdrawal of milk may have affected the growth of rabbits by an indirect effect on health, as shown by the higher mortality rate of early weaned rabbits. The higher mortality rate of early weaned rabbits presumed a higher sensitivity to digestive disturbances. This agreed with the recent results of Gallois *et al.* (2007) showing the protective effect of milk intake in the young rabbit challenged with an enteropathogenic strain of *Escherichia coli*. In addition, enteropathies (a disease of the intestine, especially the small intestine) are frequently seen during weaning of rabbits. They give rise to increased mortality rates, as well as retarded growth and consequently economic losses. However, the reasons for the high mortality rate are different, although most of the deaths are due to the stress caused by separation of weanlings from the doe and the deprivation of mother's milk. From comparative studies in rabbits weaned at different weaning ages, we can suggest that moderate (W28) weaning age had a positive effect on growth and survival/mortality rate of rabbits compared with early and later weaning age.

Intestine measurements

Little is known about the mode of action and the effect of weaning age on performance of rabbits. Effect of weaning age on digestive maturation of young rabbits is not yet completely identified. However, their knowledge is essential to determine the nutritional requirements of young rabbits around weaning, when they are more sensitive to digestive disorders.
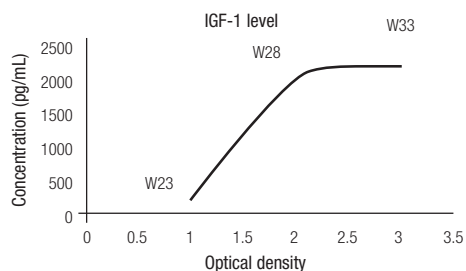
1. Intestinal morphological analysis

Morphometric parameters reflect the integral effect of all factors influencing digestive tract growth and development. Therefore, study of morphological changes in rabbit digestive tract related with weaning age is very important, especially the development of small intestine, which can influence several digestive tract functions in rabbit. In general, vital organs such as small intestine are proportionally more developed at the time of birth and, in consequence, grow proportionally less in postnatal life (Pálsson, 1955). Intestinal morphology is also markedly affected by dietary feed components and the morphological changes in the intestine correlate with intestinal function.

From our results, it seems that the small intestine length was not clearly affected by different weaning ages. Nevertheless, weaning age can result in several metabolic changes that lead to modified function of the digestive system, especially in small intestine, as well as changes in body weight and immune-depression (mortality).

A shortening of intestine was typically observed at early weaning (Pluske *et al.,* 1997) and could be explained by the anorexia provoked by the weaning, or by the withdrawal of growth factors present in milk and having a positive action on intestine growth (IGF-1). However, the morphological development of the intestine is probably implicated in the sensitivity of kits to digestive disorders. Likewise, the transition from nursing to pellet feed could be associated with considerable changes in the physiology of the digestive tract in early weaned rabbits (Kovăcs *et al.,* 2008).

2. Quantitative Real Time-PCR analysis

IGF-1 is an important factor controlling body growth and development of growing rabbits. The rabbit IGF-1 gene is more complex than that of birds (Kajimoto and Rotwein, 1989) due to splicing isoforms such as IGF-1Ea and IGF-1Eb.

Monitoring gene expression by measuring mRNA levels in tissues using quantitative RT-PCR assay is a sensitive tool to detect subtle changes in gene expression. We used qRT-PCR to measure intestinal IGF-1 mRNA level and from results we observed that weaning age affects growth of small intestine, which has a great impact on the digestive process, and then general growth and weight. We also recommend that weaning at W28 or W33 is better than at W23.

Small intestinal growth is associated with insulin-like growth factor 1 (IGF-1), which has a primary significance in the growth of the muscular wall layer of the distal part of the small intestine (Cummins and Thompson, 2002). Development of the intestinal epithelium and digestive capacity are driven by intrinsic genetic programming, but other factors, including diet, can have a significant impact on this development (Kelly and Coutts, 2000).

Evaluation of Insulin-like Growth Factor 1 in blood

Growth hormone (GH) stimulates the production of insulin-like growth factor-1 (IGF-1). IGF-1 is a hormone that mediates the GH effects and helps promote normal growth and development for tissues. Moreover, GH level is stable in the blood throughout the day and this makes IGF-1 a useful indicator of average GH levels.

ELISA assay has high sensitivity and excellent specificity for detection of rabbit IGF-1. From our results, the IGF-1 serum concentrations in rabbits are affecting weaning age. Early weaning of rabbits has a negative impact on growth. It is therefore important to be aware of the systemic physiological variations occurring, in order to decrease the impact of weaning on the further development of rabbits.

CONCLUSION

The results of this study indicated that weaning age significantly influenced growth and mortality rates of rabbits. Kits from V-line (medium-sized strain) weaned at 28 and 33 d of age had better post-weaning performance in terms of body weight and mortality rate. However, the rabbits weaned at 28 d (moderate) performed better than those weaned at 33 d, taking into account the total feed intake and physiological status of the doe. The early weaning age is not recommended at all unless specific conditions are provided for these rabbits, and then it will be more expensive. This therefore suggests that moderate (not early or late) weaning, in addition to being favourable for the farm economy, will improve rabbit production better than early or late weaning.

REFERENCES

Bivolarski B.L., Vachkova E.G., Ribarski S.S. 2011. Effect of weaning age upon the slaughter and physicochemical traits of rabbit meat. *Vet. Arhiv., 81: 499-511.*

Cummins A., Thompson F. 2002. Effect of breast milk and weaning on epithelial growth of the small intestine in humans. *Gut, 51: 748-754. doi:10.1136/gut.51.5.748*

Duclos M.J. 2005. Insulin-like growth factor-I (IGF-1) mRNA levels and chicken muscle growth. *J. Physiol. Pharmacol., 56: 25-35.*

Estany J., Baselga M., Blasco A., Camacho J. 1989. Mixed model methodology for the estimation of genetic response to selection in litter size of rabbits. *Livest. Prod. Sci., 21: 67-76. doi:10.1016/0301-6226(89)90021-3*

Feng C.Y., Von Bartheld C.S. 2011. Expression of insulin-like growth factor 1 isoforms in the rabbit oculomotor system. *Growth Horm. IGF Res., 21: 228-232. doi:10.1016/j.ghir.2011.06.001*

Feugier A., Smit M.N., Fortun-Lamothe L., Gidenne T. 2006. Fibre and protein requirement of early weaned rabbits and the interaction with weaning age: effects on digestive health and growth performance. *Anim. Sci., 82: 493-500. doi:10.1079/ASC200666*

Fonteh F.A., Niba A.T., Kudi A.C., Tchoumboue, J., Awah-Nduku J. 2005. Influence of weaning age on the growth performance and survival of weaned guinea pigs. *Livest. Res. Rural Develop.,* 17: 12.

Gallois M., Gidenne T., Fortun-Lamothe L., Le Huerou-Luron I., Lalles J.P. 2004. Weaning age and development of the small intestinal mucosa in the young rabbit. *In Proc.: 8th World Rabbit Congress, Septembre 7- 10, 2004, Puebla, Mexico, 1079-1085.*

Gallois M., Gidenne T., Tasca C., Caubet C., Coudert C., Milon A., Boullier S. 2007. Maternal milk contains antimicrobial factors that protect young rabbits from enteropathogenic *Escherichia coli* infection. *Clin. Vaccine Immunol., 14: 585-592. doi:10.1128/CVI.00468-06*

Gidenne T., Fortun-Lamothe L. 2004. Growth, health status and digestion of rabbits weaned at 23 or 32 days of age. *In Proc.: 8th World Rabbit Congress, Septembre 7- 10, 2004, Puebla, Mexico, 846-852.*

Hoon J.N., Oliver W.J., Griessel P.J. 2010. The effect of different weaning practices on post - weaning growth of Angora kids and reproduction of Angora ewes. *Grootfontein Agri. Bulletin, 8: 1.*

Kajimoto Y., Rotwein P. 1989. Structure and expression of a chicken insulin-like growth factor I precursor. *Mol. Endocrinol., 3: 1907-1917. doi:10.1210/mend-3-12-1907*

Kelly D., Coutts A.G. 2000. Development of the digestive and immunological function in neonates: role of early nutrition. *Livest. Prod. Sci., 66: 161-167. doi:10.1016/S0301-6226(00)00223-2*

Kovács M., Milisits G., Szendrő Zs., Lukács H., Bónai A., Pósa R., Tornyos G., Kovács F., Horn P. 2008. Effect of different weaning age (days 21, 28 and 35) on Caecal microflora and fermentation in rabbits. *In Proc.: 9th World Rabbit Congress, June 10-13, 2008, Verona, Italy.*

Livak K.J., Schmittgen T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. *Methods, 25: 402-408. doi:10.1006/meth.2001.1262*

Logan J., Edwards K., Saunders N.A. 2009. Real-time PCR: Current technology and applications, *Horizon Scientific Press.*

Pálsson H. 1955. Conformation and body composition. *In: Hammond, J. (Ed). Progress in the physiology of farm animals. London, UK: Butterworths, p. 575.*

Pluske J.R., Hampson D.J., Williams I.H. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livest. Prod. Sci., 51: 215-236. doi:10.1016/S0301-6226(97)00057-2*

SPSS Statistical Package for the Social Sciences 2011. SPSS User's Guide: Statistics, Version 20.0 for Windows, *Institute, Inc., Cary, NC, USA.*

Tirapegui J. 1999. Effect of insulin-like growth factor-1 (IGF-1) on muscle and bone growth in experimental models. *Int. J. Food Sci. Nutr., 50: 231-236. doi:10.1080/096374899101102*

Trocino A., Xiccato G., Sartori A., Queaque P.I. 2001. Effect of starter diet and weaning age on growth, caecal fermentation and body composition of young rabbits. *In Proc.: 2nd Meetings of Workgroup 3rd and 4th COST Action 848, Gödöllő, Hungary, 52-53.*

Tůmová E., Zita L., Marounek M., Skřivanová V., Berladyn A. 2006. The effect of weaning age on performance, nutrient digestibility and lipase activity in broiler rabbits. *In Proc.: 3rd Rabbit Congress of the Americas, August, 21-23 Maringá, Brasil.*

Xiccato G., Trocino A., Sartori A., Queaque P.I. 2003. Effect of weaning diet and weaning age on growth, body composition and caecal fermentation of young rabbits. *Anim. Sci., 77: 101-111.*

Zita L., Tumova E., Skrivanova V., Ledvinka Z. 2007. The effect of weaning age on performance and nutrient digestibility of broiler rabbits. *Czech J. Anim. Sci., 52: 341-347.*