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Evaluation of physiological parameters of plasma oxidative status in rabbits

Abstract

The evaluation of serum oxidative parameters can be a very useful tool in to assessing the status of animal health. The aim of our work is to analyze the oxidative parameters in rabbits, under physiological conditions. To allow the assessment of the animal oxidative status we measured the level of serum reactive oxygen metabolites (ROMs) and the level of serum antioxidants (d-ROMs and anti-ROMs tests respectively, Diacron s.r.l.). The levels of d-ROMs (expressed in CARR U), showed values of 299.9 ± 6.1 at the beginning of the study and of 296.8 ± 6.4 at the end of the study. The levels of anti-ROMs (fast antioxidants), expressed in μEq of reduced iron/l, showed values of $85.2 \pm 2.1 \mu\text{Eq /l}$ at the beginning of the study and of $85.4 \pm 2.7\mu\text{Eq /l}$ at the end of the study. The statistical analysis of the values, for all considered parameters, showed that the mean of the differences between the beginning and the end of the experimental period, was not significantly different from 0. These results of d-ROMs and anti-ROMs tests (Diacron s.r.l.) were obtained in rabbits under physiological conditions of feeding and rearing. Further studies are needed to assess the variations of these parameters related to oxidative stress in other physiological states of rabbits (pregnancy or weaning) or in rabbits affected by diseases.

Key words: Rabbits, oxidative stress, antioxidants.

1. Introduction

In recent years, there has been considerable attention to the preservation of livestock welfare in order to ensure the optimal growth conditions to the animals and to preserve them from multifactorial diseases that can result in a heavy impact on the zootechnical productivity (Cerioli et al. 2006; 2008; Ludwig et al. 2006; 2007; 2008; Luzi et al. 2007). The intensive breeding of rabbits in recent decades showed many problems related to the appearance of some enteric and metabolic infections that resulted in high mortality in the animals, thus weighing heavily on the productivity of farms (Luzi et al. 2007). These pathologies have multifactorial etiologies and occur at certain delicate stages of rabbits production, as a result of predisposing factors such as imbalances in the diet, hygiene deficiencies, environmental and climatic factors, overcrowding and stress (Broom and Johnson 2005, Costantini and Castellini 1990). Beside the zootechnical parameters, the evaluation of the oxidative plasmatic status in farm animals is important in monitoring animal welfare (Vassalle 2009). This assessment is carried out in order to verify whether the animal would be able to maintain a homeostatic condition in spite of stressful environmental stimuli directed to an exaltation of meat productivity (Chirase et al. 2004). According to Brambilla et al. (2003) from a prognostic point of view in the state of animal welfare, two different stressful situations, namely physiological or pathological, may be considered. In the case of physiological stress, the organism of animals is able to develop an adaptive response expressed through an activation of endogenous antioxidant mechanisms,

that can compensate the imbalance of oxidative status. Conversely, under conditions of pathological stress, the adaptive response of the organism is not adequate and leads to an excessive production of free radicals, which result in oxidative stress (Brambilla et al., 2003, Khadija et al. 2009). In fact, the excessive generation and/or inadequate removal of the free radicals result in destructive and irreversible cell damages (Lopaczynski and Zeisel 2001). Oxidative stress can be measured directly, detecting free radical production, or indirectly, detecting antioxidant defenses of the organism. Cellular antioxidant defenses consist of a complex interacting network, and more than forty molecules are involved in the oxido-reduction metabolism. (Montuschi et al. 2004, Shishehbor and Hazen 2004, Tsimikas 2008). The evaluation of the oxidative plasmatic status (the level of serum reactive oxygen metabolites - ROMs - and the level of serum antioxidant) may have several implications of veterinary interest. For instance, it can identify some negative situations inside the farm, thus suggesting the most appropriate interventions to put the animals in optimal conditions. In fact, in farm animals, oxidative stress is involved in a number of pathological conditions, including those associated with animal production, reproduction, and welfare (Lykkesfeldt and Svendsen 2007, Pastorelli et al. 2010). The evaluation of plasma oxidative level in animals would therefore result to be very useful in assessing the status of animal health (Pasquini et al., 2008; Brambilla et al., 2002). Unfortunately, the oxidative parameters in healthy rabbit, under standard conditions of nutritional and zootechnical breeding, are not well defined in the literature. To fill this gap of knowledge, we evaluated the plasma oxidative status (reactive oxygen metabolites and serum antioxidants) in rabbits fed with balanced diets for growth, and reared under standard environmental conditions (such as the size of cages and climatic conditions). To verify that rabbits were in good nutritional status serum levels of albumin and triglycerides were also evaluated as they are among the most important blood parameters of animal nutritional status. Albumins actually indicate the level of proteins in the blood, while the level of triglycerides give us some information on the metabolism of nutrients.

2. Materials and methods

2.1 Animals, diets and experimental design

Thirty growing crossbred of New Zealand female rabbits aged 70 days and weighing, on average, 2318±40 g, were housed individually in standard conditions at a temperature of 22 °C± 2 °C, at relative humidity of 70 % ± 5 and at estimate temperature - humidity index (THI, Marai et al., 2002) of about 26; in wire cages 70 x 50 cm wide at a height of 90 cm from the concrete floor. The animals were fed with a pelleted standard diet, balanced and formulated for the coverage of all the needs of rabbits, including vitamins and minerals, according to the requirements in (NRC,1977). This diet was similar to the diets used usually for the rabbits on farms, and was suitable for the physiological conditions of rabbits (NRC, 1977) (table 1). The diet was stored in darkness to avoid autooxidation of the lipid sources. The experimental period lasted 60 days. Feed and water were available ad libitum to the animals. At the end of the experimental period all the rabbits were weighed and were slaughtered according to Italian regulations (Italian Legislative Decree No. 333 of 1/9/1998: implementation of

Directive 93/119/EC).

2.2 Analytical determinations of diet.

The proximate composition of the diet was determined following AOAC procedures (AOAC, 1990). Three representative samples of the diet were taken at different times during the trial period and were used (mixed together) for the analyses. The diet was analysed to determine dry matter, organic matter, crude protein, crude fibre, ether extract (EE) (using the Soxhlet method), ash (by ignition at 550°C) and nitrogen free extract. The diet was also analysed to determine the neutral detergent fibre (NDF) without sodium sulfite or α -amylase, and acid detergent fibre (ADF), as described by Van Soest et al.(1991), expressed exclusive of residual ash, acid detergent lignin (ADL) determined by solubilization of cellulose with sulphuric acid, as described by Robertson and Van Soest (1981), and gross energy (GE) by means of an adiabatic bomb calorimeter (IKA C7000, Staufen, Germany).

2.3 Biochemical analyses of rabbit serum

Blood samples were collected in non heparinized tubes at the beginning and at the end of the experimental period. The blood samples were taken, at morning, from the ear veins of the rabbits. The blood was allowed to clot and was then centrifuged at 3000 rpm for 15 minutes at room temperature. The serum was then separated and stored at -70 °C until analyses. The analyses of albumins and triglycerides were carried out in the laboratories of the Department of Veterinary Sciences at the University of Turin, Italy. The ILab Aries analyzer (Instrumentation Laboratory, Milan, Italy) was used to analyze serum levels of albumins and triglycerides. Reactive oxygen metabolites (ROMs) were evaluated, on rabbit serum, using the d-ROMs test (Diacron s.r.l., Grosseto, Italy) (Benedetti et al. 2004, Cesarone et al. 1999, Pasquini et al. 2008). This test is based on the principle that oxygen free radicals are atoms that possess one or more unpaired electrons in one of their outer orbitals; due to their extreme reactivity, these free radicals tend to react with some organic molecule and to generate some reactive oxygen metabolites. The latter are more stable than their predecessors and can then be quantified. In the d-ROMs test, the ROMs (primarily hydroperoxides) generate (by the "Fenton reaction), in the presence of iron (released from plasma proteins by means of an acidic buffer), some alkoxy radicals and peroxy radicals. These radicals react with an aromatic amine which is oxidized and converted into a pink derivative which can be quantified photometrically, at a wavelength of 550 nm (Giongo et al., 2011) .The intensity of the color developed is directly proportional to the concentration ROMs, according to Beer-Lambert law. The results of the d-ROMs test are expressed in arbitrary units known as "Unit Carratelli" (1 CARR U = 0.08 mg hydrogen

peroxide/ dl)), according to the following formula:

$$\text{CARR U} = F(\Delta \text{ Abs} / \text{ minutes})$$

where F is a correction factor with an assigned value (approximately 9000 at 37°C, according to the results obtained with the standard), and ($\Delta \text{Abs}/\text{min}$) are the mean differences of the absorbances recorded at 1, 2, and 3 minutes.

The serum antioxidants were evaluated by the anti-ROMs test (Diacron s.r.l., Grosseto, Italy). This method exploits the ability of antioxidants to reduce ferric iron to ferrous iron, giving rise to a red-purple coloration which can be quantified photometrically at a wavelength of 550 nm, due to the reaction with the molecule of $\alpha\alpha'$ -dipyridyl. Color intensity increases proportionally according to the quantity of iron reduced by the antioxidants present in the sample (Giongo et al., 2011.) This test enables discrimination between the concentration of the so-called "fast antioxidants", determined at the start by the instrument, i.e. those which are fast-acting, such as Vitamin C or Vitamin E, and the concentration of "slow antioxidants", subsequently determined by the instrument, such as thiol-SH groups and uric acid. Results are expressed in μEq of reduced iron/liter using ascorbic acid as a standard according to Giongo et al. (2011). To ensure the accuracy and sensitivity of these tests we used diagnostic kits and a spectrophotometer calibrated on purpose by the Diacron s.r.l., Grosseto, Italy.

2.4 Statistical analyses

In order to establish whether the mean differences between the measures on the blood samples collected at the beginning and at the end of the experimental phase is significantly different from zero, we carried out paired difference test. Given the relatively high number (30) of individuals in the samples, a Wilcoxon signed-rank test (≤ 0.01) was performed only in presence of strong violations of the Shapiro-Wilk normality test (≤ 0.01) on the differences between the groups. In the absence of strong violations of this assumption, a paired t-test (≤ 0.01) was employed in the statistical analyses of the samples. All analyses were performed using R statistical analysis software, version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

In our study the rabbits were reared in standard conditions and the diets were formulated in order to satisfy the nutritional requirements of growing rabbits. The ingredients of the diet, the chemical composition and gross energy of the diet are reported and shown in Table 1. The zootechnical performances of rabbits (initial live weight, final live weight, total feed consumption, total weight gain and feed efficiency values) are given in Table 2. In our study the productive performances are generally in agreement with other nutritional studies in rabbits

(Alikata et al. 1992, Dal Bosco et al. 2004, Yalçın et al. 2003). The biochemical analyses of rabbit serum are reported in Table 3. The levels of albumins and triglycerides at the beginning and at the end of the study did not show a mean difference significantly different from zero (paired t-test with p-value equal to 0.356 and 0.886 for albumins and triglycerides, respectively). Furthermore the values of albumin and triglycerides lie in a standard range for rabbits (Kaneko et al. 2008); this may indicate that the rabbits were within physiological nutritional conditions, both for proteins and plasma lipids (Table 3). Actually, the level and quality of protein and lipid contained in the diets influence the plasma albumins and triglycerides; if these parameters are not in the reference range for rabbits, this may mean a dietetic imbalance. Indeed, the protein-energy malnutrition status (PEM) is indicated by hypoalbuminemia. Albumin is synthesized by liver, and serum albumin is a major component of serum proteins, which sustains osmotic pressure. Serum albumin is the most common index of nutrition status (Sekine et al., 2013). Its concentration in serum is also influenced by many factors that are independent from nutritional factors, such as infections, trauma (by an increase in the transcapillary escape rate of albumin), hydration status (by haemodilution), liver function (by an increase in synthesis) and kidney disease (by albumin losses). (Sekine et al., 2013). Furthermore, the influence of diet on plasma triglycerides concentrations is a subject of great interest. Elevated levels of plasma triglycerides may be due to a state of dyslipidemia related to dietary imbalances. The most common cause of elevated triglyceride levels or dyslipidemia is undoubtedly the overnutrition. However the ingestion of more calories than are needed not only increases the adipose tissue, but also promotes triglycerides synthesis by the liver. The different types of fatty acids differently affect the serum triglycerides; in fact medium-chain saturates fatty acids have been reported to increase triglycerides, instead the polyunsaturates fatty acids, have been reported to reduce serum triglycerides in some patients with hypertriglyceridemia (Scott et al., 1990).

Since rabbits clinically showed a good health during the trial we did not other blood analysis in addition to albumins and triglycerides considered as nutritional parameters.

Table 4 collects the descriptive values of ROMs and antioxidants in rabbit serum at the beginning and at the end of the experiment, expressed as mean \pm standard error of the mean.

In our study, the levels of d-ROMs (expressed in CARR U) were 299.9 ± 6.1 at the beginning of the study and 296.8 ± 6.4 at the end of the study. Also for these parameters, the statistical analyses did not find a mean difference significantly different from zero (paired t-test with p-value equal to 0.705). Therefore these values did not change during the study period. Although the d-ROMs method has been successfully tested “in vitro” and applied in some animal species (swine, dog) and in humans, the reference values in rabbits are lacking (Giongo et al. 2011). For example, in researches on humans, the reference value of the d-ROMs test, determined on a sample of about 5.000 healthy men is between 250 and 300 CARR U (corresponding to the range between 20.00

and 24.00 mg hydrogen peroxide/dl), regardless of gender and age. However, infants have values significantly lower, while pregnant women higher. In humans values greater than 300 CARR U indicate progressively increasing levels of oxidative stress. The values of d-ROMs that we found in rabbits are almost similar to those seen in human; in other animal species, on the contrary, the values of d-ROMs were different.

Brambilla et al. (2002), for instance, have considered the response to oxidative stress an effective parameter for assessment of welfare in pigs; in their study carried out in pigs the values of d-ROMs are around between 550-600 CARR U (Ballerini et al. 2003, Brambilla et al. 2002). Furthermore, the normal reference of the d-ROMs test in dogs was ranging between 56.4 and 91.4 CARR U (Pasquini et al. 2008).

As far as the assessment of the plasma antioxidants is concerned, we found values of anti-ROMs test (fast antioxidants, in particular vitamin E and vitamin C) ranging between $85.2 \pm 2.1 \mu\text{Eq/l}$ at the beginning of the study and $85.4 \pm 2.7 \mu\text{Eq/l}$ at the end of the study. The values of anti-ROMs test (slow antioxidants, such as thiol-SH groups) have a range between $594.7 \pm 4.2 \mu\text{Eq/l}$ at the beginning of the study and $597.2 \pm 3.5 \mu\text{Eq/l}$ at the end of the study. For these parameters too, the statistical analyses did not find a mean difference significantly different from zero (paired t-test with p-value equal to 0.941 and 0.636 for fast and slow antioxidants, respectively). The anti-Roms parameters in healthy rabbit, under standard conditions of nutritional and zootechnical breeding, have not yet been defined in the literature. In humans, however, the values of anti-ROMs test were different from those that we found in rabbits. Actually in healthy humans, values greater than 200 micro-equivalents/L are considered optimal, for the first result (relating to rapid antioxidants) and values greater than 1000 micro-equivalenti/L are considered optimal, for the second result (relating to slow antioxidants). Values lower than those limits are indicative of a condition of oxidative stress, by reduced antioxidant defenses. This will be useful to investigate the possible causes which led the the lowering of one or both values (Giongo et al., 2011).

The separated tests (fast and slow antioxidants) provide clearer indications that those resulting from a measurement of the total antioxidant capacity of plasma.. (Giongo et al., 2011).

In our study, both the values of the d-ROMs test and anti-ROMs test were unchanged during the test phase; being the conditions of the experimental period similar for all animals, we calculated the confidence interval of the values at the end of the experimental period (Table 5).

The evaluation of plasma oxidative status for the different species of animals could become an important parameter of animals health condition (Vassalle 2009). Until now the available techniques to estimate the value of the oxidative state, presented some technical characteristics that limited their use to specialized research laboratories (Griendling and Fitzgerald 2003, Ridker et al. 2004). Recently faster, cheaper, reliable, and easier

techniques have been developed and proposed (Ridker et al. 2004, Vassalle and Andreassi 2004). Some of these tests may also be implemented with automated analyzers allowing to a simultaneous execution of large number of samples in a short time, avoiding the handling of the sample and further reducing the sources of variability compared to manual protocols (Vassalle 2009). The evaluation of oxidative stress lends to various applications of veterinary interest by pointing out negative events that could affect the welfare of the animal at a farm level. Actually in the assessment of animal welfare, the increase of reactive oxygen metabolites (ROMs) that is not counterbalanced by an adequate antioxidant response, may reflect a stress exposure due, for example, to poor transport conditions (the long trips, the overcrowding, the high temperature, the thirst) (Vassalle and Andreassi 2004, Vassalle et al. 2009). Moreover, the parameters of oxidative stress can act as biomarkers for the detection of illicit hormonal treatments that affects the safety and quality of animal products for human consumption. In conclusion, we evaluated the parameters of plasma oxidative status in rabbits under physiological conditions; thus providing an initial approach to the knowledge of these parameters in rabbits as it was done, by other authors, in other animal species. Further investigations are needed, in a long period, in different rearing conditions and in a more complex physiological state (pregnancy, weaning) or in rabbits affected by diseases in which these parameters of oxidative stress could be more elevated .

References

- Alikata ML, Bomanno A, Alabiso M, Portolano B, Stimolo MC. 1992. Further trials on the use of chick-peas in growing rabbit feeding. *J. Appl. Rabbit Res.* 15:1025–1032.
- AOAC. 1990. Official Method of Analysis. In: Cuniff P. (Ed.), 15th ed., AOAC International, Arlington, VA, USA.
- Ballerini A, Civitareale C, Fiori M, Regini M, Betti M, Brambilla G. 2003. Traceability of inbred and crossbred Cinta Senese pigs by evaluating the oxidative stress. *Journal of Veterinary Medicine Series A.* 50(3): 113-116.
- Benedetti S, Lamorgese A, Piersantelli M, Pagliarani S, Benvenuti F, Canestrari F. 2004. Oxidative stress and antioxidant status in patients undergoing prolonged exposure to hyperbaric oxygen. *Clinical biochemistry.* 37(4):312-317.
- Brambilla G. 2003. Genetica, selezione e benessere animale. In: 5° Congresso Nazionale MultisalaSivar. Cremona, 30-31 maggio 2003.
- Brambilla G, Ballerini A, Civitareale C, Fiori M, Neri B, Cavallina R, Nardoni A, Giannetti L. 2003. Oxidative stress as a bio-marker of estrogen exposure in healthy veal calves. *Anal Chim Acta.* 483: 281-288.
- Brambilla G, Civitareale C, Ballerini A, Fiori M, Amadori M, Archetti L, Betti M. 2002. Response to oxidative stress as a welfare parameter in swine. *Redox Report.* 7(3): 159-163.
- Broom DM, Johnson KG. 1993. Stress and animal welfare. Chapman & Hall, London, UK.
- Cerioli M, Brivio R, Salogni C, Grilli G, Lavazza A. 2006. Valutazione dello stato zoosanitario e immunitario per la individuazione di parametri “in campo” del benessere del coniglio allevato. *Riv. Coniglicoltura.* 46(4):18-19.
- Cerioli M, Grilli G, Brivio R, Martino PA, Lavazza A. 2008. Salute, ambiente e benessere del coniglio in allevamento: quali novità? Lodi (Italy) Convegno ASIC, pp. 13-20.
- Cesarone MR, Belcaro G, Carratelli M, Cornelli U, De Sanctis MT, Incandela L, Barsotti A, Terranova R, Nicolaidis. 1999. A simple test to monitor oxidative stress. *International Angiology : a Journal of the International Union of Angiology.* 18(2):127-130.
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- Costantini F, Castellini C. 1990. Effetti dell'habitat e del management. *Rivista di Coniglicoltura.* 27(2): 31-36.
- Dal Bosco A, Castellini C, Bianchi L, Mugnai C. 2004. Effect of dietary α -linolenic acid and vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit meat. *Meat Sci.* 66:407-413.
- Giongo L, Bozza E, Caciagli P, Valente E, Pasquazzo MT, Pedrolli C, Costa A. 2011. Short-term blueberry intake enhances biological antioxidant potential and modulates inflammation markers in overweight and obese children. *Journal of Berry Research.* 1(3):147-158.
- Griendling KK, FitzGerald GA. 2003. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. *Circulation.* 108:1912-1916.
- Kaneko JJ, Harvey JW, Bruss ML. 2008. *Clinical Biochemistry of Domestic Animals.* Sixth edition. Academic Press, p. 916.
- Khadija A, Ati A, Mohammed S, Saad AM, Mohamed HE. 2009. Response of broiler chicks to dietary monosodium glutamate. *Pak. Vet. J.* 29(4):165-168.
- Lopaczynski W, Zeisel SH. 2001. Antioxidants, programmed cell death, and cancer. *Nutrition Research.* 21(1):295-307
- Ludwig N, Gargano M, Luzi F, Carezzi C, Verga M. 2006. Applicability of I.R. thermography to the measurement of stress in rabbit. In: Proc. 8th Int. Conf. Quant. Infra Red Therm., Padova, Italy.
- Ludwig N, Gargano M, Luzi F, Carezzi C, Verga M. 2007. Applicability of infrared thermography as a non invasive measurement of stress in rabbits. *World Rabbit Sci.* 15:199-206.
- Ludwig N, Gargano M, Redaelli V, Luzi F, Verga M. 2008. Analisi di immagini termiche per la valutazione del benessere animale: uno studio quadriennale sulla specie cunicola presso l'Università degli studi di Milano. Lodi: Convegno ASIC, pp. 21-28.
- Luzi F, Verga M, Martino AM, Dusanka J, Stuhec I, Szendro Z. 2007. Come si può migliorare il benessere in allevamento. *Riv. Coniglicoltura.* 44(2):28-31.

- Lykkesfeldt, J, Svendsen, O. 2007. Oxidants and antioxidants in disease: oxidative stress in farm animals. *The Veterinary Journal*. 173(3):502-511.
- Marai IFM, Habeeb AAM, Gad AE. 2002. Rabbit ĩs productive, reproductive and physiological performance traits as affected by heat stress: a review. *Livestock Production Science*. 78: 71–90.
- Montuschi P, Barnes PJ, Roberts LJ. 2004. Isoprostanes: markers and mediators of oxidative stress. *The FASEB Journal*. 18(15):1791-1800.
- NRC. National research council. 1977. Nutrient requirements of rabbits. Subcommittee on rabbit nutrition, Committee on animal nutrition. Board on agriculture and renewable resources. 2. rev. ed., Washington : National academy of sciences.
- Pasquini A, Luchetti E, Marchetti V, Cardini G, Iorio EL. 2008. Analytical performances of d-ROMs and BAP test in canine plasma. Definition of the normal range in healt Labrador dogs. *Vet Res Commun*. 32:137-143.
- Pastorelli G, Rossi R, Cannata S, Corino C. 2010. Total antiradical activity in male castrated piglets' blood: reference values. *Italian Journal of Animal Science*. 8(2s):640-642.
- Ridker PM, Brown NJ, Vaughan DE, Harrison DG, Mehta JL. 2004. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. *Circulation*. 109:IV6–IV19.
- Robertson JB, Van Soest PJ. 1981. The detergent system of analysis. In: James, W.P.T., Theander, O. (Eds.), *The Analysis of Dietary Fibre in Food*. Marcel Dekker, NY, p. 123–158.
- Shishehbor MH, Hazen SL. 2004. Antioxidant studies need a change of direction. *Cleveland Clinic journal of medicine*. 71(4):285-288.
- Tsimikas S. 2008. In vivo markers of oxidative stress and therapeutic interventions. *The American journal of cardiology*. 101(10): S34-S42.
- Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci*. 74: 3583–3591.
- Vassalle C, Andreassi MG. 2004. 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease. *Circulation*. 110:49–50.
- Vassalle C. 2009. An easy and reliable automated method to estimate oxidative stress in the clinical

setting. In: Armstrong D, Ed. Advanced Protocols for Oxidative Stress in the Methods in Molecular Biology series. Vol. 477. pp. 31-9.

Yalçin S, Tuncer I, Yalçin S, Onbaşilar EE. 2003. The use of different levels of common vetch seed (*Vicia sativa* L.) in diets for fattening rabbits. *Livest. Prod. Sci.* 84: 93–97.