RESEARCH PAPER | Zoology | Volume : 3 | Issue : 12 | Dec 2013 | ISSN - 2249-555X

Effects of hen's egg white proteins on reproductive performance of red flour beetle, *Tribolium castaneum* (Coleoptera : Tenebrionidae)

KEYWORDS: Eggs, Egg white Proteins, Ovarioles, Oogenesis, Reproductive performance, *Tribolium castaneum*

Geetu Choudhary	Megha Kansal
Department of Zoology, Punjab Agricultural University, Ludhiana 141 004, India	Department of Zoology, Punjab Agricultural University, Ludhiana 141 004, India.

ABSTRACT *Tribolium castaneum is a destructive pest of stored grains and is renowned for adapting to various insecticides. The hen's egg white proteins have been shown to possess unique insecticidal properties. Therefore, the present study was carried out to determine the effect of egg proteins on the reproductive parameters of T. castaneum with protein samples obtained through salt fractionation (PPT-I and PPT-II) and whole egg white (WEW) were used after lyophilization. Different concentrations (2%, 3%, 4%, 5%, 10%) of each of the protein samples were tested by adding them to wheat flour and milk powder (75:25 w/w). Marked abnormalities were observed in appearance and dimensions of ovarioles and eggs which indicated that the egg protein samples caused considerable effect on the process of oogenesis. SEM studies revealed changes in the structure of epithelial sheath cells of ovarioles probably caused due to resorption of ova in the beetles fed on PPT-II proteins. Higher resolution of the egg surface showed the presence of wrinkles or shriveled appearance of the chorionic layer in PPT-I and PPT-II treated eggs and these deep folds and wrinkles were more conspicuous in the beetles fed on PPT-II proteins. Therefore, PPT-II proteins appeared to be most effective in causing reduction of fecundity in T.castaneum.*

1. Introduction

The red flour beetle, *T.castaneum* (Herbst) is a serious pest of stored grain and grain based products in many parts of the world. The use of various insecticides such as malathion, pirimiphos-methyl, chlorpyrifos-methyl, deltamethrin and the fumigant phosphine, are the currently used products against stored-product pests. However these synthetic insecticides are the source of serious health and ecological problems, such as development of pest strains resistant to pesticides (Riebeiro et al 2003), toxicity to mammals and detection of residues in human food (Bugiho & Wilkin, 2004). These problems oriented investigators towards the research of other alternative eco-friendly methods.

The proteins of egg white include ovalbumin, ovotransferrin, ovomucoid, lysozyme, ovoglobulins, ovostatin, avidin, thiamine binding proteins and glutamylaminopeptidase (Awade, 1996). These proteins have also proven to be of great significance in agriculture because of their potential to affect growth and survival of various pathogens and pests (Kizek et al 2005). A molar excess of avidin in an insect diet causes deficiency in accessible biotin, resulting in abnormal development and even death in a number of insects (Kramer et al 2000 & Masarik et al 2003). The gene coding for avidin production has been cloned and inserted into maize and potato providing resistance to a wide spectrum of insect pests (Kramer et al 2000 & Cooper et al 2006). Kramer et al. (2000) reported that avidin is also toxic to the housefly (*Musca domestica* Linnaeus), hide beetle (*Dermeste maculates* De Geer), olive fruit fly (*Dacus olea* Gmelin), fruit fly (*Drosophila melanogaster* Meigen), flour mite (*Acarus siro* Linnaeus), European corn borer (*Ostrinia nubilaliso* Hubner) and tobacco horn-worm (*Manduca Sexta* Linnaeus). There is extensive evidence of antibacterial effects of ovotransferrin and lactoferrin, based on iron deprivation property, as iron is an essential growth factor for most of the microorganisms (Awade, 1996). Studying the effect of hen's egg white proteins on *T.castaneum* female ovarioles and eggs indicated that the size of the treated eggs and ovarioles were markedly affected by the concentration and duration of the exposed doses. So, the aim of the current study was to explore the effect of Hen's egg white proteins on reproductive performance of red flour beetle, *Tribolium castaneum.*

2. Materials and methods

2.1 Insect stock culture

Cultures of *T.castaneum* were maintained by placing beetles in sterilized glass jars containing sterilized wheat flour\milk powder mixture (75:25 w\w). The colonies were reared under experimental conditions (30±1°C, 75±5% RH).

2.2 Preparation of Egg White Protein Samples

Whole egg white (WEW) and two protein fractions (PPT-I and PPT-II) were obtained through salt precipitation method (Bernardi & Cook, 1960) and then these protein fractions were used after freeze drying.

2.2.1 WEW: Pricked the shell of the egg carefully and the egg white was taken out in a flask slowly and was lyophilized till it got dried.

2.2.2 PPT-I: Carefully removed the shell of another egg and collected its egg white in 100ml beaker. Then 1:1 dilution of the collected egg white was made with distilled water and equal volume of saturated ammonium sulphate was added with gentle stirring and frothing was avoided while stirring. Centrifuged at 2500- 3000rpm for 10min. Precipitates formed were separated from the supernatant and the latter was decanted in a test tube and was lyophilized till it got dried.

2.2.3 PPT-II: The supernatant decanted above was saturated by addition of solid ammonium sulphate with constant stirring. Centrifuged at 2500- 3000rpm for 10min. Precipitates formed were separated from the supernatant and were collected in a test tube and was lyophilized till it got dried.

2.3 Beetle Dissection and Measurement of Eggs and Ovarioles

Sterilized glass bottles were taken and sterilized 10g of rearing medium was added in each bottle. Then 2%, 3%, 4%, 5%, 10% of each of the three protein samples (i.e. WEW, PPT-I, PPT-II) were added and simultaneously control with 10g of rearing medium and without egg proteins were run. Then 50 beetles in each bottle were released and the mouth of each bottle was covered with muslin cloth and the bottles were kept in the incubator at 30°C ± 1°C and 95% RH. After 30 days of treatment equal number of beetles were dissected from each treatment including control under dissecting microscope in a drop of Ringer's physiological solution on a wax-fixed petridish. A pair of surgical pins was used to open the abdominal cavity and then the ovarioles were taken out (Anwar et al 1971). The surrounding tracheoles and fat bodies were removed. The size of ovarioles and eggs were taken with the help of occular micrometer precalibrated with stage micrometer.

2.4 Morphometeric measurements of eggs and ovarioles
Females were sampled from control and treated series after 30 days of treatment and the size of ovarioles were taken with of ocular micrometer pre-calibrated with stage micrometer.

2.5 Determination of fecundity, egg volume and reproductive effort per female
The total number of eggs laid by each female, i.e., the fecundity, the egg volume [length (a) and breadth (b) of the eggs were measured using ocular micrometer pre-calibrated with stage micrometer, and the volume (V) was calculated using the formula $V=\pi ab^2$, on the assumption that the shape ellipsoid (Fujiwara et al 2000) and the reproductive effort per female (Fecundity x Egg volume) (Yin et al 2008) was compared between control and treated groups.

2.6 Scanning Electron Microscopic study
The ovarioles and eggs of female specimens after 30 days of feeding of treated and untreated rearing medium were removed and fixed in 2.5% glutaraldehyde for two hours and post fixation was done in osmium tetraoxide for an hour followed by three buffer washings of 15 minutes each after each fixation to fixation. The tissues were then dehydrated in a graded ethanol series (50%-100%) for 15-20minutes in each grade. After drying, the samples were assembled on aluminium stubs, coated with gold and were examined for SEM (Almeida & Landim, 2000).

2.7 Data analysis
The data was presented as mean ± SE and were analysed using Analysis of Variance (ANOVA) in order to determine the significant differences in control and treated groups**.**

3. Results and discussion
3.1 Normal structure of the ovary
The female reproductive system in *T.castaneum* is of the meroistic\telotrophic type in which nurse cells, or trophocytes, are present in the germarium and are connected to oocytes in early stages of their development by trophic filaments called nutritive cords (King & Buning, 1985). In the beetle, this cord is extremely slender, less than 10 µm in diameter. At the time of yolk uptake, the nutritive cord finally breaks and the follicle cells form a complete layer round the oocyte (Chapman, 1998). There was a pair of ovaries with 5-6 ovarioles each, generally aligned parallel to one another. Each ovariole contains a terminal filament. The average length of each ovariole of female *T.castaneum* is about 0.67 mm (Table 1).Each ovary has a lateral oviduct that ends at the common oviduct which opens into a genital chamber called bursa copulatrix. Even though there must be a separation between the common oviduct and the vagina, the later was not differentiated in *T.castaneum*. At the dorsal side of the base of the vagina, the accessory organs were observed, which were the spermatheca and one accessory gland. Female accessory gland supply lubricants for the reproductive system and secrete a protein-rich egg shell (chorion) that surrounds the entire egg. These glands were usually connected by small ducts to the common oviduct or the bursa copulatrix.

The ovarioles were divided into two main regions: the more distal and somewhat enlarged germarium in which oocytes are produced from oogonia, and a more proximal vitellarium in which oocytes grow and mature (Plate1-Fig.1). The vitellarium contained a series of 2-3 follicles (oocytes with a surrounding follicular epithelium) in successive stages of development (Plate 1-Fig.1). All the ovarioles present in *T.castaneum* were asynchronous, since there were not mature oocytes at the same time.

3.2 Effects of treatment on ovarioles
The sizes (length and breadth) of ovarioles of normal mature red flour beetles and the beetles treated with different concentrations (2%, 3%, 4%, 5%, 10%) of egg white protein samples (WEW, PPT-I and PPT-II) were compared (Table 1) after 30 days of treatment. The average length of ovariole of a normal control beetle was about 0.67mm while that of the treated beetles were found to be ranged between 0.27mm to 0.61mm, and breadth of the normal ovariole of the control beetle was about 0.10 to 0.11mm and that of the treated beetles were ranging between 0.08 to 0.10mm. So it was observed that the length of the ovariole was significantly less in the beetles fed on 5% and 10% WEW; 4%, 5% and 10% PPT- I and 2%, 3%, 4%, 5% and 10% PPT-II. The breadth of the ovariole of all the treated beetles was significantly less except in 4% PPT- I and 4% PPT- II as compared to the control beetles. Ovarioles from treated female red flour beetles had also some abnormal oocytes as compared to the control ones. Some ovarioles were malformed, while the others in a state of deterioration being resorbed (Plate 1-Figs. 2,3,4). The degenerating eggs lost their spherical shape without separating boundaries between adjacent eggs in the beetles fed on the diet containing WEW, PPT- I, PPT- II. The continuous intake of this egg white protein samples (WEW, PPT- I, PPT- II) also caused early death of the insects as compared to the normal untreated ones.

3.3 Fecundity, egg volume and reproductive effort per female
The fecundity rate of the beetles were significantly ($p<0.05$) lower in 3%, 4%, 5% and 10% of both PPT-I and PPT-II than that of the control group (approximately 10.5) after 30 days of treatment. In these treated beetles, fecundity rate ranged from 0.25 to 3.5 (Fig.1 a). The mean value of egg volume was 0.84 x 10-2 mm3 in the control group whereas the mean value of egg volume of the beetles varied from 0.69 to 0.81x10-2 mm3 in WEW, 0.12 to 0.64 x 10-2 mm3 in PPT-I and 0.64 to 0.76 x 10-2 mm3 in PPT-II (Fig.1 b). So out of all the three treatment proteins (WEW, PPT-I, PPT-II), PPT-I proteins had maximum and WEW had minimum effect on the egg volume (Fig.1 b). The mean reproductive effort of the control individual was 0.085 whereas the mean reproductive effort of the female adults emerging from treatment and control lots exhibited a highly appreciable difference. The mean value of reproductive effort of the beetles reared on the diet containing WEW varied from 0.013 to 0.068 in different concentration groups (2%, 3%, 4%, 5%, 10%) but it was significantly less only in the beetles reared on higher concentrations i.e., 5% and 10% WEW as compare to the control group (Fig.1 c). The mean value of reproductive effort of the beetles fed on the diet containing 2%, 3%, 4%, 5%, and 10% of PPT-I ranged as 0.053, 0.005, 0.008, 0.002 and 0.001 respectively. These values were significantly very less than the control group except in the beetles having 2% PPT-I. However, in the beetles fed on 3%, 4%, 5%, 10% PPT-II protein, the values of reproductive effort were also significantly smaller than those of the control group except in 2% PPT-II (Fig.1 c). So the different egg white proteins (WEW, PPT-I, PPT-II) had shown an inhibitory effects on the reproduction of insects. They could reduce survival rate, fecundity and net reproductive rate of *T.castaneum* which predicts that the egg white proteins are likely to reduce the general fitness of an insect and thus the reproductive capacity.

3.4 Scanning Electron Microscopy of ovariole surface
The changes in shape, appearance and size of ovarioles in treated females as compare to the control group indicated that hen's egg white proteins may have considerable effect on development of ovary/ovariole. This could be supported by the data of fecundity and egg size which decreased in treated female beetles as compare to that of control (Fig.1). Higher

Volume : 3 | Issue : 12 | Dec 2013 | ISSN - 2249-555X

resolution of the ovariole surface revealed the presence of wrinkled appearance which marks the ovariole sheath. There was found a markable change in the structure of the ovariole sheath of control and treated groups (Plate 2-Figs. 1,2,3,4). In the control beetles, in the germarium region of ovariole, the epithelial sheath cells were flat and could be visualized as juxtaposed "disks" (Plate 2-Fig. 1). In the beetles fed on the diet containing WEW and PPT-I, the epithelial sheath was quite similar to the control group though some changes in these juxtaposed disks were reported but in case of the beetles fed on the diet containing PPT-II proteins, the epithelial sheath cells were remarkably changed showing some sort of resorption in the ovariole sheath. The epithelial sheath of the treated beetles (i.e, WEW, PPT-I, PPT-II), in the middle and posterior region of vitellaria, also showed remarkable change in the structure of sheath as compare to the control group (Plate 2-Figs.2,4).

3.5 Scanning Electron Microscopy of egg surface

Eggs presented polygonal ridges on the external surface of the chorion and these were particularly distinct (Plate 3-Fig.1). The exochorion is made up of the two sheets, which trap a film of air between them and functions as a plastron in the respiratory system of insect eggs. (Hinton, 1970 & Margaritis & Mazzini, 1998). The small, scattered aeropyles were also seen on the surface of Tribolium eggs (Plate 3-Fig.2). These aeropyles enable the eggs to transfer atmospheric oxygen through the plastron network. Micropylar region was seen on the top of the egg in a rounded shape. Higher resolution of the egg surface revealed the presence of wrinkled \ shrivelled appearance of the chorionic layer on the beetles fed on the PPT-I and PPT-II proteins respectively (Plate 3-Figs. 3, 4). So formation of deep wrinkles and folds in the chorionic layer of eggs points towards the depletion of internal cellular material.

4. CONCLUSION

The results of the study have shown that feeding of the egg white proteins to larval and adult stages of *Tribolium castaneum* caused significant effect on the survival/mortality and the reproductive performance. Fecundity rate appeared to be reduced due to the effect of various protein fractions on oogenesis and egg characteristics. Similar to the results of this study it was observed that all the protein fractions of egg white cause mortality and disrupt testicular functions in red flour beetle. Fraction PPT-II appeared to be more effective in inducing mortality and degenerative changes in the testis than the whole egg white proteins and PPT-I (Parshad & Kansal, 2012). Higher rate of mortality of beetles observed in PPT-II fraction may be due to avidin, which is expected to be present in this fraction, however, further studies need to be carried out to ascertain the biopesticidal properties of egg white proteins.

ACKNOWLEDGEMENTS

Thanks are due to Dr. Gursharan Singh, Professor of Entomology, Dr. KS. Khera, Professor of Zoology and Dr. Anil Gupta, Professor and Head, Department of Biochemistry, Punjab Agricultural University, Ludhiana for their help in carrying out the present studies.

Table 1: Measurement of ovariole dimensions in red flour beetle reared on medium without or with variable concentrations of different egg white proteins.

Treatment	% protein	Size of ovarioles (mm)	
		Length	Breadth
Control	0	0.67±0.05	0 .11±0.01
Whole egg white	2	0.45±0.06*	0.08±0*
	3	0.43±0.03*	0 .08±0*
	4	0.61±0.02	0.08±0*
	5	0.45±0.05*	0.08±0*
	10	0.40±0.08*	0.09±0.006*
PPT - I	2	0.73±0.04	0.09±0.006*
	3	0.57±0.02	0.08±0.10*
	4	0.42±0.06*	0.10±0.10
	5	0.34±0.07*	0.09±0.005*
	10	0.34±0.03*	0.09±0.006*
PPT - II	2	0.27±0.10*	0.08±0*
	3	0.42±0.02*	0.08±0*
	4	0.42±0.04*	0.10±0*
	5	0.38±0.03*	0.08±0*
	10	0.31±0.02*	0.08±0*

Values are mean ± SE

* Significant differences ($p<0.05$) between control and treatment groups.

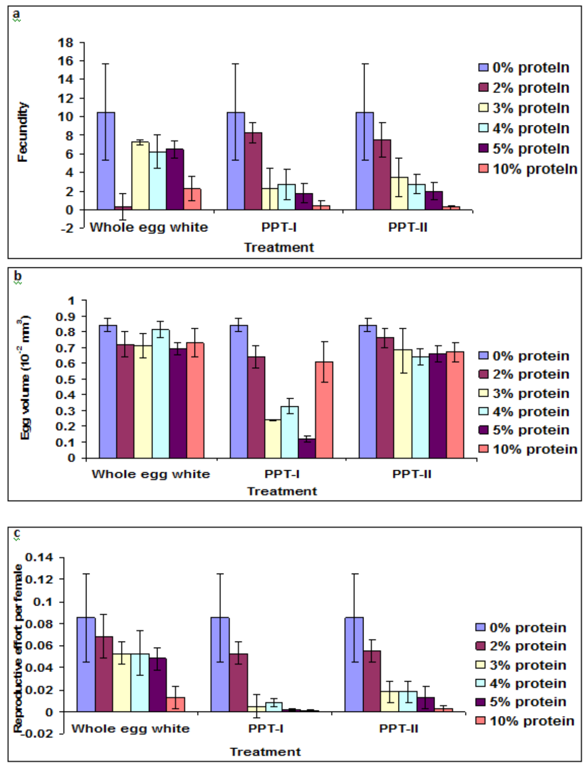
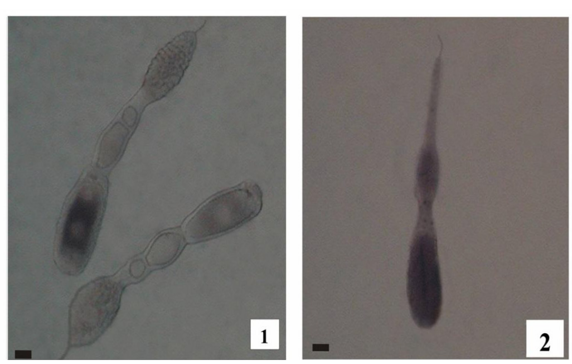


Fig. 1 : Fecundity, egg volume and reproductive effort per female in red flour beetle reared on medium without or with variable concentrations of different egg white proteins.



Volume : 3 | Issue : 12 | Dec 2013 | ISSN - 2249-555X

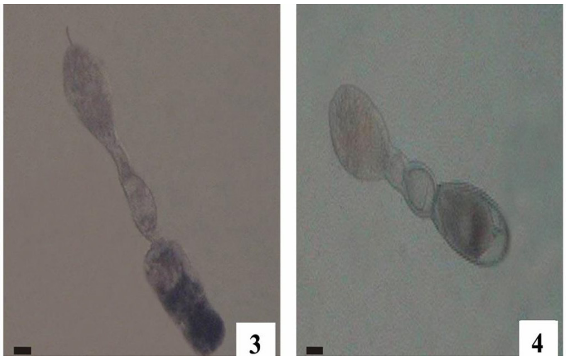


PLATE-1

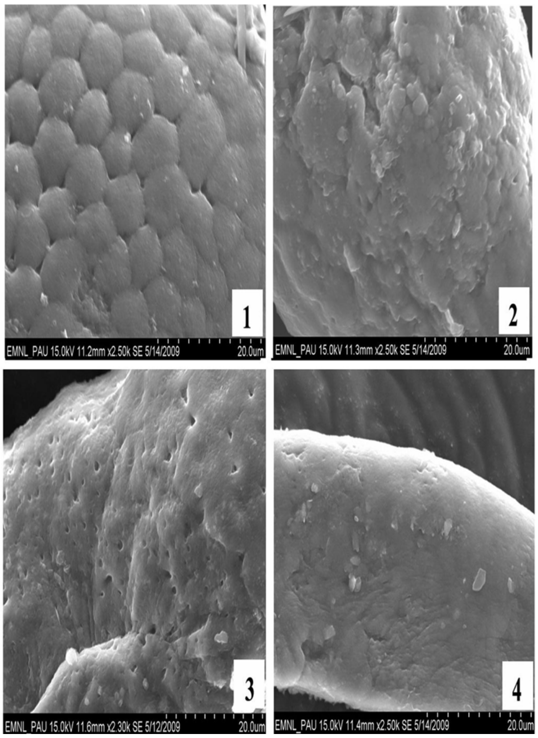


PLATE-2

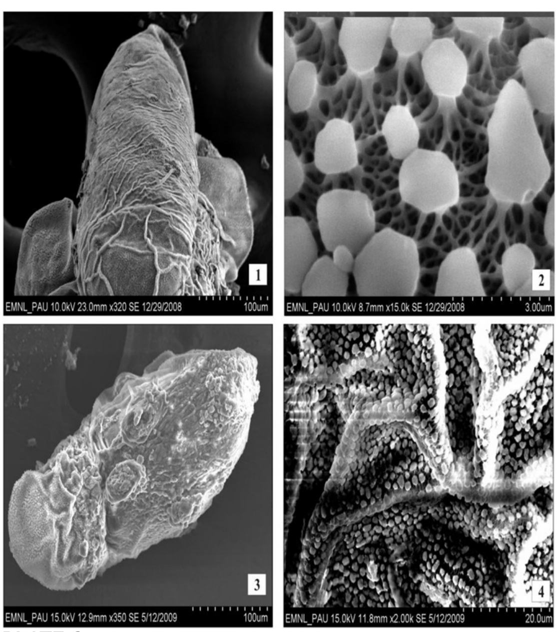


PLATE-3

Legends: Explanation of photographs

Plate 1
Fig. 1: Light photomicrograph of ovarioles of beetles reared on control medium. X 100

Fig. 2: Abnormality in the germarium region of ovariole of beetle fed on rearing medium containing treatment. X 100

Fig. 3: Abnormality in the vitellarium region of ovariole of beetle fed on rearing medium containing treatment. X 100

Fig. 4: An ovariole with oosorptive oocyte of beetle fed on rearing medium containing treatment. X 100

The bar indicated the scale of 100μm
Plate 2
Fig. 1: Epithelial sheath of anterior germarium region of ovariole of beetles fed on control medium.

Fig. 2: Epithelial sheath of anterior germarium region of ovariole of beetles fed on PPT-II.

Fig. 3: Epithelial sheath of posterior vitellarium region of ovariole of beetles fed on control medium.

Fig. 4: Epithelial sheath of posterior vitellarium region of ovariole of beetles fed on PPT-II.

Plate 3
Fig. 1: Scanning electron micrographs of egg surface of the beetle fed on control medium.

Fig. 2: Higher resolution micrograph of the aeropyles on the egg surface.

Fig. 3: Scanning electron micrographs of egg surface of the beetle fed on PPT-II.

Fig. 4: Deep folds and wrinkles on the egg surface of beetle reared on the rearing medium containing PPT-II.

REFERENCE

Beever, J. E., Fisher, S. R., Guerin, G., Lewin, H. A., 1997. Mapping of eight human Chromosome 1 orthologs to cattle Chromosomes 3 and 16. Mammalian Genome. 8, 533-536. | Berkes, C. A., Tapscott, S. J., 2005. MyoD and the transcriptional control of myogenesis. Seminars in Cell & Developmental Biology. 16, 585-595. | Bhuiyan, M. S. A., Kim, N. K., Cho, Y. M., Yoon, D., Kim, K. S., Jeon, J. T., Lee, J. H., 2009. Identification of SNPs in MYOD gene family and their associations with carcass traits in cattle. Livestock Science. 126, 292-297. | Bilal, M. Q., Suleman, M., Raziq, A., 2006. Buffalo: Black gold of Pakistan. Livestock Research for Rural Development. 18 (9). | Chu, M., Yan, P., Liang, C.-n., Guo, X., Pei, J., Ding, X.-z., Bao, P.-j., Zhu, X.-s., 2012. Association between Polymorphism of MyoD1 Gene and Growth Traits in Yak. China Animal Husbandry & Veterinary Medicine. 2. | Cieslak, D., Kapelanski, W., Blicharski, T., Pierzchala, M., 2000. Restriction fragment length polymorphisms in myogenin and myf3 genes and their influence on lean meat content in pigs. Journal of Animal Breeding and Genetics-Zeitschrift Fur Tierzuchtung Und Zuchtungsbiologie. 117, 43-55. | Ensembl database, 2013. Ensembl release 73 - September 2013. Cow Myogenin [online]. Available from http://www.ensembl.org/Bos_taurus/Gene/Summary?db=core;g=ENSBTAG00000006030;r=16:596920-599757;t=ENSBTAT00000007923 [accessed 14 November 2013]. | Fatima, S., Bhatt, S. M., Bhong, C. D., Rank, D. N., Joshi, C. G., 2009. Genetic Polymorphism Study of Igf-I Gene in Buffaloes of Gujarat. Buffalo Bulletin. 28, 159-164. | Franzolin, R., 1994. Feed efficiency: a comparison between cattle and buffalo. Buffalo J.supplement 2, 39-50. | Ishido, M., Kami, K., Masuhara, M., 2004. Localization of MyoD, myogenin and cell cycle regulatory factors in hypertrophying rat skeletal muscles. Acta physiologica Scandinavica. 180, 281-289. | Kapelanski, W., Grajewska, S., Kuryl, J., Bocian, M., Wyszynska-Koko, J., Urbanski, P., 2005. Polymorphism in coding and non-coding regions of the MyoD gene family and meat quality in pigs. Folia Biologica-Krakow. 53, 45-49. | Kim, J. M., Choi, B. D., Kim, B. C., Park, S. S., Hong, K. C., 2009. Associations of the variation in the porcine myogenin gene with muscle fibre characteristics, lean meat production and meat quality traits. Journal of Animal Breeding and Genetics. 126, 134-141. | Lee, E. A., Kim, J. M., Lim, K. S., Ryu, Y. C., Jeon, W. M., Hong, K. C., 2012. Effects of variation in porcine MYOD1 gene on muscle fiber characteristics, lean meat production, and meat quality traits. Meat Science. 92, 36-43. | Liu, M., Peng, J., Xu, D.-Q., Zheng, R., Li, F.-E., Li, J.-L., Zuo, B., Lei, M.-G., Xiong, Y.-Z., Deng, C.-Y., Jiang, S.-W., 2008. Association of MYF5 and MYOD1 Gene Polymorphisms and Meat Quality Traits in Large White × Meishan F2 Pig Populations. Biochemical Genetics. 46, 720-732. | Liu, Z. Z., Gong, Y. F., Zhang, C. S., Fu, Z. X., Zhang, W. X., 2011. A genetic polymorphism and its genetic effects on goat myogenin gene in intron 1. African Journal of Biotechnology. 10, 5263-5267. | Michelizzi, V. N., Dodson, M. V., Pan, Z. X., Amaral, M. E. J., Michal, J. J., McLean, D. J., Womack, J. E., Jiang, Z. H., 2010. Water Buffalo Genome Science Comes of Age. International Journal of Biological Sciences. 6, 333-349. | Paul, S. S., Mandal, A. B., Kannan, A., Mandal, G. P., Pathak, N. N., 2003. Comparative dry matter intake and nutrient utilisation efficiency in lactating cattle and buffaloes. Journal of the Science of Food and Agriculture. 83, 258-267. | Rejduch, B., 2008. Genes associated with production and health in farm animals. Journal of Central European Agriculture. 9 No. 4, 829-836. | Ryan, A. M., Schelling, C. P., Womack, J. E., Gallagher, D. S., Jr., 1997. Chromosomal assignment of six muscle-specific genes in cattle. Animal Genetics. 28, 84-87. | Song, X.-c., Yang, F.-h., Xing, X.-m., Liu, H.-t., Yue, Z.-g., 2010. Detection of Single Nucleotide Polymorphisms and DNA Sequence Variation Analysis on 5' UTR of MyoG Gene in Gansu Wap (Cervus Elaphus Kansuensis). China Animal Husbandry & Veterinary Medicine. 9. | Soumillion, A., Erkens, J. H., Lenstra, J. A., Rettenberger, G., te Pas, M. F., 1997. Genetic variation in the porcine myogenin gene locus. Mammalian Genome. 8, 564-568. | Spanghero, M., Gracco, L., Valusso, R., Piasentier, E., 2004. In vivo performance, slaughtering traits and meat quality of bovine (Italian Simmental) and buffalo (Italian Mediterranean) bulls. Livestock Production Science. 91, 129-141. | Strazzullo, M., Rossetti, C., Fusco, G., Campanile, C., Vecchio, D., Campanile, G., Perucatti, A., Di Meo, G. P., Filippini, F., Eggen, A., Ferrara, L., D'Esposito, M., 2010. Genomic characterization and chromosomal mapping of 5 river buffalo skeletal muscle differentiation master genes. Cytogenetic and Genome Research. 128, 221-227. | Stupka, R., Citek, J., Sprysl, M., Okrouhla, M., Brzobohaty, L., 2012. The impact of MYOG, MYF6 and MYOD1 genes on meat quality traits in crossbred pigs. African Journal of Biotechnology. 11, 15405-15409. | Sung, M. S., Mun, J.-Y., Kwon, O., Kwon, K.-S., Oh, D.-B., 2013. Efficient myogenic differentiation of human adipose-derived stem cells by the transduction of engineered MyoD protein. Biochemical and Biophysical Research Communications. 437, 156-161. | Tapscott, S. J., 2005. The circuitry of a master switch: Myod and the regulation of skeletal muscle gene transcription. Development. 132, 2685-2695. | te Pas, M. F., Soumillion, A., Harders, F. L., Verburg, F. J., van den Bosch, T. J., Galesloot, P., Meuwissen, T. H., 1999. Influences of myogenin genotypes on birth weight, growth rate, carcass weight, backfat thickness, and lean weight of pigs. Journal of Animal Science. 77, 2352-2356. | te Pas, M. F. W., 2004. Candidate genes for meat production and meat quality – the MRF genes. Animal Science Papers and Reports. 22, 115-118. | Tian, L., Xu, S. Z., Yue, W. B., Li, J. Y., Gao, X., Ren, H. Y., 2007. Relationship between genotypes at MyoD locus and carcass traits in cattle. Yi Chuan. 29, 313-318. | Ujan, J. A., Zan, L. S., Wang, H. B., Ujan, S. A., Adoligbe, C., Wang, H. C., Biao, S. F., 2011a. Lack of an association between a single nucleotide polymorphism in the bovine myogenic determination 1 (MyoD1) gene and meat quality traits in indigenous Chinese cattle breeds. Genetics and Molecular Research. 10, 2213-2222. | Ujan, J. A., Zan, L. S., Wei, S. J., Adoligbe, C., Wang, H. B., 2011b. Meat tenderness and water holding capacity are associated with a 959 A -> G mutation in the MyoG gene of Chinese indigenous cattle. African Journal of Biotechnology. 10, 5654-5660. | Urbanski, P., Kapelanski, W., Wyszynska-Koko, J., Bocian, M., Pierzchala, M., Kuryl, J., 2006. An association between the MyoD gene polymorphisms and carcass traits in two- and three-breed crossbred pigs. Animal Science Papers and Reports. 24, 297-303. | Verner, J., Humpolicek, P., Knoll, A., 2007. Impact of MYOD family genes on pork traits in Large White and Landrace pigs. Journal of Animal Breeding and Genetics-Zeitschrift Fur Tierzuchtung Und Zuchtungsbiologie. 124, 81-85. | Wang, M., Su, P., Liu, X., Zhao, Z., Wu, S., Zhang, Z., Li, Q., 2011. Single Nucleotide Polymorphic Analysis of MyoG Gene in Cattle,Yak and Water Buffalo. Acta Agriculturae Boreali-Occidentalis Sinica. 6, 7-11. | Wu, Y., Pi, J. S., Pan, A. L., Pu, Y. J., Du, J. P., Shen, J., Liang, Z. H., Zhang, J. R., 2012. An SNP in the MyoD1 Gene Intron 2 Associated with Growth and Carcass Traits in Three Duck Populations. Biochemical Genetics. 50, 898-907. | Xue, H.-L., Zhou, Z.-X., 2006. Effects of the MyoG Gene on the Partial Growth Traits in Pigs. Acta Genetica Sinica. 33, 992-997. | Xue, M., Zan, L. S., Gao, L., Wang, H. B., 2011. A novel polymorphism of the myogenin gene is associated with body measurement traits in native Chinese breeds. Genetics and Molecular Research. 10, 2721-2728. | Yin, H. D., Zhang, Z. C., Lan, X., Zhao, X. L., Wang, Y., Zhu, Q., 2011. Association of MyF5, MyF6 and MyOG Gene Polymorphisms with Carcass Traits in Chinese Meat Type Quality Chicken Populations. Journal of Animal and Veterinary Advances. 10, 704-708. | Zhang, H. J., Chen, H., Fang, X. T., Zhang, R. F., Bao, B., Gao, X. Y., Shao, R. Y., 2007. Association of variations in MyoD family of genes with body measurement traits in goat. Yi Chuan. 29, 1077-1082. |