Original Article Buffalo Bulletin (April-June 2023) Vol.42 No.2
https://doi.org/10.56825/bufbu.2023.4225257

THE MILK IGF-2 LEVEL IS POSITIVELY CORRELATED WITH MILK YIELD IN ANATOLIAN WATER BUFFALOES

Hakan Guler[1], Gul Fatma Yarim[2,*], Murat Yarim[3], Ozkan Duru[4] and Ayris Gokceoglu[2]

Received: 26 December 2022
Accepted: 27 June 2023

ABSTRACT

The aim of this study was to investigate the relationship between milk insulin-like growth factor-2 (IGF-2) concentration and milk yield in Anatolian water buffaloes. This study was conducted on milk samples from 80 Anatolian water buffaloes. Milk samples collected from buffaloes were evaluated for subclinical mastitis. For this purpose, the California mastitis test and somatic cell count were performed in milk samples taken from four different mammary lobes of buffaloes. The milk IGF-2 level was determined by an enzyme-linked immunosorbent assay kit. Milk IGF-2 concentration ranged from 21.6 ng/ml - 63.2 ng/ml in Anatolian water buffaloes. The IGF-2 concentration in Anatolian water buffaloes milk was 40.1±8.5 ng/ml. Milk IGF-2 was positively correlated with milk yield (r^2=0.941, P<0.01). Our results showed that milk IGF-2 concentration was associated with milk yield in Anatolian buffaloes. These findings show that locally synthesized IGF-2 can affect milk yield. This study contributes to the understanding of the composition of buffalo milk, which has an important value in human nutrition. It is recommended to confirm the results of similar measurements in milk from other animal species used for human consumption.

Keywords: *Bubabus bubalis*, buffaloes, Anatolian water buffalo, insulin-like growth factor-2, milk yield

INTRODUCTION

Milk is the healthiest food for every newborn, which can meet its nutritional needs differ according to yield and composition. Milk is an excellent source of well-balanced nutrients and also exhibits a range of biological activities that affect digestion, metabolic responses to absorbed nutrients, growth and development of certain organs, and resistance to disease. Breed,

[1]Department of Animal Health Economics and Management, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey
[2]Department of Biochemistry, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey, *E-mail: gulyarim@omu.edu.tr
[3]Department of Pathology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey
[4]Department of Biochemistry, Faculty of Veterinary Medicine, Kirikkale University, Kirikkale, Turkey

diet, lactation stage, frequency and method of milking, as well as meteorological conditions, all have an impact on the quantity of milk's bioactive components (Tripaldi and Palocci, 2008). In recent years, the use of buffalo milk in human nutrition is gaining increasing popularity due to its therapeutic properties (Colarow *et al*., 2003; D'Onofrio *et al*., 2019). Buffalo milk is considered excellent because of its valuable nutrient profile (Li *et al*., 2020). When compared to the milk of other animal species, buffalo milk has a number of significant advantages. High dry matter and fat content compared to cow's milk increase the demand for buffalo milk in dairy products technology (Salman *et al*., 2014; Arrichiello *et al*., 2022). In addition to traditional laboratory methods, scientific studies on the level of components in milk have gained momentum in order to monitor udder health in dairy herds and to determine the quality of raw milk (Sadek *et al*., 2017; Simões *et al*., 2018; De Matteis *et al*., 2021; Raj *et al*., 2021). The relationship between the content of buffalo milk and milk yield has been reported (El-Moghazy *et al*., 2015).

Insulin-like growth factor-2 (IGF-2), a mitogenic peptide hormone influences the activities of numerous tissues, including the mammary gland, ovary, skeletal muscle, adipose tissue, and bone (Hovey *et al*., 2003; Fiedler *et al*., 2006; Wilson *et al*., 2006; Spicer and Aad, 2007; Wang *et al*., 2019). IGF-2 is primarily produced by the liver in adults, but it is also produced by numerous other tissues, where it is then released into the pericellular fluid. Additionally, other abundant sources are the placenta and the fetus (Nielsen, 1992). Significant fetal growth retardation occurs in IGF-2 knockout mice, particularly in the first several weeks of pregnancy In mice, during sexual maturity and early pregnancy, the level of IGF-2 mRNA in the mammary gland is increased simultaneously with prolactin receptor expression (Baker *et al*., 1993). IGF-2 regulates cell growth, differentiation, and metabolism through endocrine, autocrine, and paracrine effects (Morali *et al*., 2000; Piecewicz *et al*., 2012).

The non-hormonal factors such as growth factors may be effective on the milk composition. It is well documented that growth factors have an impact on milk yield (Grochowska *et al*., 2001). IGF-2 plays a role in postnatal growth and differentiation of the mammary gland (Plath-Gabler *et al*., 2001). Locally produced IGF-2 has been proven to mediate the action of prolactin, which causes an increase in alveolar formation, ductal branching, and alveolar development in the mammary gland (Hovey *et al*., 2003). IGF-2 is a potential candidate gene for quantitative trait loci (QTL) affecting milk production (Ron *et al*., 2007). The relationship between IGF-1/SnaBI gene polymorphism and milk production and milk components in dairy cattle has been reported (Bonakdar *et al*., 2010). There are limited reports of IGF-2 levels in mother's milk (Elmlinger *et al*., 2007; Milsom *et al*., 2008; Goelz *et al*., 2009) and cow's milk (Kang *et al*., 2007). The level of IGF-2 in milk, which is a valuable human food, is very important for human health because IGF-2 is linked to conditions such as diabetes, obesity, and cancer. Though the relationship between IGF-1 and milk yield was demonstrated (Taylor *et al*., 2004; Szewczuk *et al*., 2013), the possible role of IGF-2 on milk yield was limited investigatedTo the best of our knowledge, this study is the first to assess the IGF-2 milk concentration and its connection to milk production in Anatolian water buffaloes.

MATERIALS AND METHODS

Collection of milk samples

This study was conducted on milk samples from 80 Anatolian water buffaloes from Samsun, a city on the north coast of Turkey from April to October 2021. Anatolian water buffaloes were 3 to 8 years old and in the middle of their lactation period. Individual milk samples from each buffalo were collected during the morning milking. Milk yield from all individual milkings of the buffaloes involved in the study was recorded daily.

Milk samples collected from buffaloes were evaluated for subclinical mastitis. For this purpose, following the California mastitis test (CMT), somatic cell count (SCC) was applied to milk samples taken from four different mammary lobes of buffaloes. The CMT test was performed according to the method reported by Schalm *et al.* (1971). Milking was performed from 4 separate udder lobes of buffaloes into the CMT test cup, and after equalizing the amount of milk in the chambers, CMT solution was added (milk/CMT; 1/1) and the color and consistency change was scored as +, ++, and +++ by rotating in circular motions.

Somatic cell counting in raw milk was performed by the microscopic method (Kilicoglu *et al.*, 1989). According to this method, milk samples taken into 10 ml glass tubes were centrifuged at 1550 g for 10 minutes, and the top milk cream was removed, then tubes were kept upside down in the port tube for 20 minutes. The sediment at the bottom of the tubes was taken on the slide with the help of a loop and physiological saline was dropped. After the preparations were dried at room temperature, they were stained with 0.2% toluidine blue and the cells in 15 to 20 microscope fields were counted in an immersion lens by dripping cedar oil. The mean cell number was determined by dividing the counted cell area by the number of fields, and the number of somatic cells in 1 ml of milk was calculated.

IGF-2 measurement

The milk IGF-2 level was measured by an enzyme-linked immunosorbent assay (ELISA) kit (MBS734487, MyBioSource, Inc. San Diego, CA, USA) following the manufacturer's protocol. The sensitivity of the kit was 0.1 ng/ml, the range was from 10 to 250 ng/ml. Briefly, balance solution of 10 µl dispensed milk samples of 100 µl/well or standards of 100 µl/well. Phosphate-buffered saline of 100 µl (pH 7.0 to 7.2) were pipetted in the blank control wells. Except for controls, 50 µl of conjugate were added to wells were incubated with for 1 h at 37^{o}C. Substrates A and B volume of 50 µl were pipetted to each well after five washes, and then incubated for 15 minutes at 37^{o}C in dark. After 50 µl of stop solution was ppetted to each well, and the absorbance of microplate was recorded in a microplate reader (Infinite F50, Tecan Austria GmbH, Grödig, Austria). The milk IGF-2 concentrations were determined using the standard curve. The intra- and interassay coefficients of variation were 7.7 and 3.0%, respectively.

Statistical evaluation

Statistical analysis was performed in the statistical package program (SPSS Statistics V22.0, IBM Corporation, Armonk, NY). Data was presented as mean±standard deviation (SD). To evaluate normality of data Shapiro-Wilks test was used. Following, Levene's test was performed to test for homogeneity of variances. Correlations between milk IGF-2 concentration and milk yield was calculated by Spearman's correlation test. A P-value of <0.05 was considered significant.

RESULTS

Daily milk yield in Anatolian water buffaloes was in the range of 2 to 9 l/day and an average of 5.9±1.7 l/day. Box-and-Whisker plot of the milk yield of the Anatolian water buffaloes was presented in Figure 1.

The milk IGF-2 level in Anatolian water buffaloes was in the range of 21.6 to 63.2 ng/ml and an average of 40.1±8.5 ng/ml. Box-and-Whisker plot of the milk IGF-2 concentration of the Anatolian water buffaloes was presented in Figure 2.

A positive correlation between the milk yield and the milk IGF-2 concentration was determined ($r^2=0.941$, $P<0.01$). A scatterplot of correlations between milk yield and milk IGF-2 concentration of the Anatolian water buffaloes was presented in Figure 3.

DISCUSSION

Buffaloes contribute significantly to total milk production in the world. Increasing interest in buffalo milk and its products has allowed buffalo production to approach the dairy industry. There is increasing interest in research in buffalo milk due to its bioactive substance content and health benefits. As the therapeutic potential of the bioactive compounds in buffalo milk is proven, interest in buffalo milk is increasing all over the world (Colarow *et al*., 2003; Servillo *et al*., 2018; D'Onofrio *et al*., 2019; Tatullo *et al*., 2022). Compared with fermented cow's milk products, those made with buffalo milk have been found to show better bacterial viability *in vitro* gastrointestinal digestion, and buffalo milk has been reported to have a potential protective effect on the human microbiome (da Silva *et al*., 2020). Buffalo milk has many important superior and advantageous properties compared to the milk of other animal species and is considered an excellent food due to its valuable nutritional profile (Colarow *et al*., 2003; Salman *et al*., 2014; D'Onofrio *et al*., 2019; Li *et al*., 2020; Arrichiello *et al*., 2022). Although the role of growth factors on cow milk yield is recognized, growth factor levels and their effects on milk yield in the buffalo milk are limited. The present study is the first report evaluating the relationship between milk IGF-2 concentration and milk yield in Anatolian water buffaloes.

IGF-2 is one of the peptide growth factors proven to play a role in mammary gland development and is expressed in developing mammary glands of both IGF-2 and its primary signal receptor, IGF-IR (Forsyth *et al*., 1999; Allar and Wood, 2004). Locally produced IGF-2 has been reported to mediate the effect of prolactin, which induces an increase in alveolar formation, ductal branching, and alveolar development in the mammary gland (Hovey *et al*., 2003). IGFBP-2 has an important function during postnatal growth and differentiation of mammary epithelium (Allar and Wood, 2004). Locally secreted IGF-2 is known to mediate mammogenesis (Plath-Gabler *et al*., 2001). In mouse primary mammary epithelial cells, IGF-2 has been shown to mediate prolactin-related morphogenesis in the mammary epithelium (Brisken *et al*., 2002). The level of IGF-2 in mother's milk 4 to 9 months after birth is 10 to 25 ng/ml has been determined (Elmlinger *et al*., 2007; Milsom *et al*., 2008). The average level of IGF-2 in the milk of the mothers who were between the 5 to 46th of their lactation days was 34.14 ng/ml has been recorded (Goelz *et al*., 2009). In a study conducted on 70 Holstein cows on two separate farms in Kyoung-ki province, milk IGF-2 concentrations ranged from

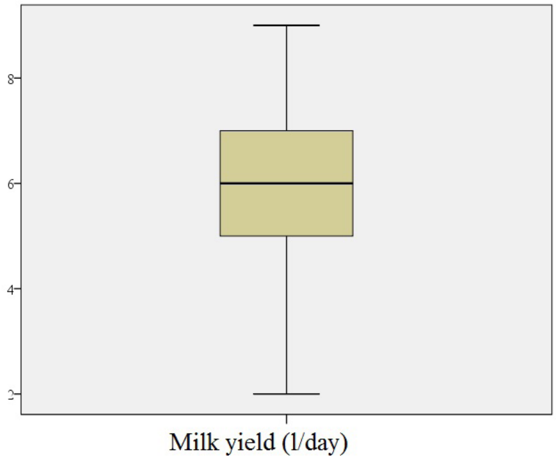

Figure 1. Box-and-Whisker plot of the milk yields of the Anatolian water buffaloes (n= 80).

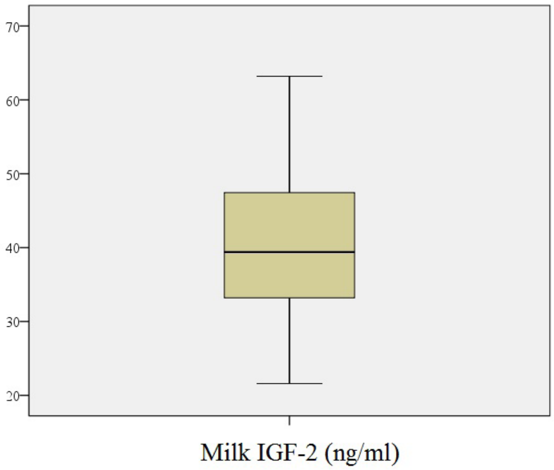

Figure 2. Concentration of milk IGF-2 in Anatolian water buffaloes (n=80).

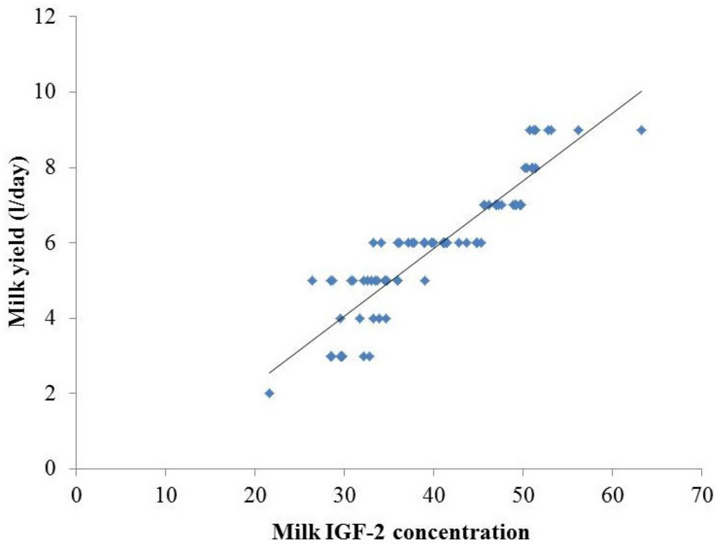

Figure 3. Scatterplot of correlations between milk yield and milk IGF-2 concentration of the Anatolian water buffaloes (n=80). Spearman's correlation test (r^2=0.941, P<0.01).

118.5 to 137.7 ng/ml on one farm and 140.7 to 165.6 ng/ml on the other farm, and these levels are not affected by the lactation period has been reported (Kang *et al*., 2007).

IGF2 plays a role in various types of carcinogenesis, and genetic and epigenetic factors are responsible for IGF-2 overexpression (Nosho *et al*., 2004). It has been suggested that the synergistic crosstalk signaling of IGF-2 with estrogen receptors may promote breast cancer progression independent of estrogen and provide new therapeutic targets for the treatment of breast cancer patients (Richardson *et al*., 2011). In addition, inter-individual genetic variations in IGF-2 have been associated with body weight and obesity (Roth *et al*., 2002; Faienza *et al*., 2010). Considering that IGF-2 plays a role in various types of cancer and obesity, the amount of IGF-2 obtained from food is important for health. Although IGF-2 has mitogenic effects on the mammary gland, ovary, skeletal muscle, adipose tissue, and bone, it is necessary to be careful about the intake of exogenous IGF-2 in terms of obesity and various cancer risks. In our study, it was determined that the IGF-2 level of Anatolian buffaloes milk (40.1±8.5 ng/ml) in the middle of the lactation period was very close to the level in mother's milk (Elmlinger *et al*., 2007; Milsom *et al*., 2008; Goelz *et al*., 2009), and quite low compared to cow's milk (Kang *et al*., 2007). Our findings indicate that Anatolian buffalo milk is a safer food than cow's milk in terms of IGF-2 levels.

Growth factors are associated with milk yield. Holstein-Friesian dairy cows with higher peak milk yield have been reported to have lower plasma IGF-1 concentrations (Taylor *et al*., 2004). According to these researchers, the level of IGF-1 in cows' milk peaked in the first week following calving, dropped sharply the next month, and did not rise again. IGF-1/SnaBI gene polymorphism affects milk production and milk components in dairy cattle (Bonakdar *et al*., 2010). Our study's results show a strong relationship between the milk IGF-2 level and daily milk production in Anatolian water buffaloes (r^2=0.941, P<0.01). A positive relationship between IGF-2 with milk yield has been reported previously. IGF-2 genotype has been reported that predictive of milk yield and composition in Polish Holstein-Friesian cattle (Bagnicka *et al*., 2010). IGF-2 is known to be a local regulator of lactogenesis. The local infusion of IGF-2 increases milk synthesis in goats has been reported (Prosser *et al*., 1994).

In this study, we determined that the level of IGF-2 in Anatolian buffalo milk is close to breast milk. This finding shows that buffalo milk is good food in terms of IGF-2 level, both in infant feeding, in childhood, and/or throughout life. In addition, milk IGF-2 level had a positively correlated with milk yield in Anatolian water buffaloes. This indicates the stimulating effect of locally produced IGF-2 in mammary tissue on milk production. Mammary epithelial cell-derived IGF-2 may contribute to this effect by acting in an autocrine or paracrine manner on mammary gland development. It is recommended that this study be approved with similar analyzes in the milk of other dairy animals and other buffalo breeds.

REFERENCES

Allar, M.A. and T.L. Wood. 2004. Expression of the insulin-like growth factor binding proteins during postnatal development of the murine mammary gland. *Endocrinology*, **145**(5): 2467-2477. DOI: 10.1210/en.2003-1641

Arrichiello, A., G. Auriemma and F. Sarubbi. 2022. Comparison of nutritional value of different ruminant milks in human nutrition. *International Journal of Functional Nutrition*, **3**(4): 1-10. DOI: 10.3892/ijfn.2022.28

Bagnicka, E., E. Siadkowska, N. Strzałkowska, B. Zelazowska, K. Flisikowski, J. Krzyzewski and L. Zwierzchowski. 2010. Association of polymorphisms in exons 2 and 10 of the insulin-like growth factor 2 (IGF2) gene with milk production traits in Polish Holstein-Friesian cattle. *J. Dairy Res.*, **1**: 37-42. DOI: 10.1017/S0022029909990197

Baker, J., J.P. Liu, E.J. Robertson and A. Efstratiadis. 1993 Role of insulin-like growth factors in embryonic and postnatal growth. *Cell*, **75**(1): 73-82. DOI: 10.1016/S0092-8674(05)80085-6

Bonakdar, E., H.R. Rahmani, M.A. Edriss and B.E.S. Tabatabaei. 2010. IGF-I gene polymorphism, but not its blood concentration, is associated with milk fat and protein in Holstein dairy cows. *Genet. Mol. Res.*, **9**(3): 1726-1734. DOI: 10.4238/vol9-3gmr874

Brisken, C., A. Ayyannan, C. Nguyen, A. Heineman, F. Reinhardt, J. Tan, S.K. Dey, G.P. Dotto and R.A. Weinberg. 2002. IGF-2 is a mediator of prolactin-induced morphogenesis in the breast. *Dev. Cell*, **3**(6): 877-887. DOI: 10.1016/S1534-5807(02)00365-9

Colarow, L., M. Turini, S. Teneberg and A. Berger. 2003. Characterization and biological activity of gangliosides in buffalo milk. *Biochim. Biophys. Acta*, **1631**(1): 94-106. DOI: 10.1016/S1388-1981(02)00360-8

De Matteis, G., F. Grandoni, M. Zampieri, A. Reale and M.C. Scatà. 2021. New insights into the significance of parp-1 activation: flow cytometric detection of poly (ADP-Ribose) as a marker of bovine intramammary infection. *Cells*, **10**(3): 599. DOI: 10.3390/cells10030599

D'Onofrio, N., A. Balestrieri, G. Neglia, A. Monaco, M. Tatullo, R. Casale, A. Limone, M.L. Balestrieri and G. Campanile. 2019. Antioxidant and anti-inflammatory activities of buffalo milk δ-Valerobetaine. *J. Agr. Food Chem.*, **67**(6): 1702-1710. DOI: 10.1021/acs.jafc.8b07166

Elmlinger, M.W., F. Hochhaus, A. Loui, K.W. Frommer, M. Obladen and M.B. Ranke. 2007. Insulin-like growth factors and binding proteins in early milk from mothers of preterm and term infants. *Horm. Res.*, 6**8**(3): 124-131. DOI: 10.1159/000100488

El-Moghazy, M.M., M.N.F. Hamad and A.A. El-Raghi. 2015. The relation between milk yield and composition of individual buffaloes. *Egyptian Journal of Dairy Science*, **43**: 141-145.

Faienza, M.F., N. Santoro, R. Lauciello, R. Calabrò, L. Giordani, G. Di Salvo, A. Ventura, M. Delvecchio, L. Perrone, E.M. Del Giudice and L. Cavallo. 2010. IGF2 gene variants and risk of hypertension in obese children and adolescents. *Pediatr. Res.*, **67**(4): 340-344. DOI: 10.1203/PDR.0b013e3181d22757

Fiedler, J., C. Brill, W.F. Blum and R.E. Brenner. 2006. IGF-I and IGF-II stimulate directed cell migration of bone-marrow-derived human mesenchymal progenitor cells. *Biochem. Bioph. Res. Co.*, **345**(3): 1177-1183. DOI: 10.1016/j.bbrc.2006.05.034

Forsyth, I.A., G. Gabai and G. Morgan. 1999. Spatial and temporal expression of insulin-like growth factor-I, insulin-like growth factor-II and the insulin-like growth factor-I receptor in the sheep fetal mammary gland. *J. Dairy Res.*, **66**(1): 35-44. DOI: 10.1017/S0022029998003240

Goelz, R., E. Hihn, K. Hamprecht, K. Dietz, G. Jahn, C. Poets and M. Elmlinger. 2009. Effects of different CMV-heat-inactivation-methods on growth factors in human breast milk. *Pediatr. Res.*, **65**(4): 458-461. DOI: 10.1203/PDR.0b013e3181991f18

Grochowska, R., P. Sørensen, L. Zwierzchowski, M. Snochowski and P. Løvendahl. 2001. Genetic variation in stimulated GH release and in IGF-I of young dairy cattle and their associations with the leucine/valine polymorphism in the GH gene. *J. Anim. Sci.*, **79**: 470-476. DOI: 10.2527/2001.792470x

Hovey, R.C., J. Harris, D.L. Hadsell, A.V. Lee, C.J. Ormandy and B.K. Vonderhaar. 2003. Local insulin-like growth factor-II mediates prolactin-induced mammary gland development. *Mol. Endocrinol.*, **17**(3): 460-471. DOI: 10.1210/me.2002-0214

Kang, S.H., J.U. Kim, Y. Kim, K.S. Han, W.J. Lee, J.Y. Imm and S.H. Kim. 2007. Changes in the levels of insulin-like growth factors (IGF-I and IGF-II) in bovine milk according to the lactation period and parity. *Asian-Austral. J. Anim. Sci.*, **20**(1): 119-123. DOI: 10.5713/ajas.2007.119

Kilicoglu, C., E. Alacam, H. Izgur, O. Akay and H.U. Wiesner. 1989. Eutergesundheitskontrolle von Milchkülen im Gebiet von Ankara (Turkei). *Deut. Tierärztl. Woch.*, **96**: 486-488.

Li, Z., S. Lu, K. Cui, L. Shafique, S.U. Rehman, C. Luo, Z. Wang, J. Ruan, Q. Qian and Q. Liu 2020. Fatty acid biosynthesis and transcriptional regulation of Stearoyl-CoA

Desaturase 1 (SCD1) in buffalo milk. *BMC Genet.*, **21**(1): 23. DOI: 10.1186/s12863-020-0829-6

Milsom, S.R., W.F. Blum and A.J. Gunn. 2008. Temporal changes in insulin-like growth factors I and II and in insulin-like growth factor binding proteins 1, 2, and 3 in human milk. *Horm. Res.*, **69**(5): 307-311. DOI: 10.1159/000114863

Morali, O.G., A. Jouneau, K.J. McLaughlin, J.P. Thiery and L. Larue. 2000. IGF-II promotes mesoderm formation. *Dev. Biol.*, **227**(1): 133-145. DOI: 10.1006/dbio.2000.9875

Nielsen, F.C. 1992. The molecular and cellular biology of insulin-like growth factor II. *Progress in Growth Factor Research*, **4**(3): 257-290. DOI: 10.1016/0955-2235(92)90023-B

Nosho, K., H. Yamamoto, H. Taniguchi, Y. Adachi, Y. Yoshida, Y. Arimura, T. Endo, Y. Hinoda and K. Imai. 2004. Interplay of insulin-like growth factor-II, insulin-like growth factor-I, insulin-like growth factor-I receptor, COX-2, and matrix metalloproteinase-7, play key roles in the early stage of colorectal carcinogenesis. *Clin. Cancer Res.*, **10**(23): 7950-7957. DOI: 10.1158/1078-0432.CCR-04-0875

Piecewicz, S.M., A. Pandey, B. Roy, S.H. Xiang, B.R. Zetter and S. Sengupta. 2012. Insulin-like growth factors promote vasculogenesis in embryonic stem cells. *PLOS One*, **7**(2): e32191. DOI: 10.1371/journal.pone.0032191

Plath-Gabler, A., C. Gabler, F. Sinowatz, B. Berisha and D. Schams. 2001. The expression of the IGF family and GH receptor in the bovine mammary gland. *J. Endocrinol.*, **168**(1): 39-48. DOI: 10.1677/joe.0.1680039

Prosser, C.G., S.R. Davis, V.C. Farr, L.G. Moore and P.D. Gluckman. 1994. Effects of close-arterial (external pudic) infusion of insulin-like growth factor-II on milk yield and mammary blood flow in lactating goats. *J. Endocrinol.*, **142**(1): 93-99. DOI: 10.1677/joe.0.1420093

Raj, A., V. Kulangara, T.P. Vareed, D.P. Melepat, L. Chattothayil and S. Chullipparambil. 2021. Variations in the levels of acute-phase proteins and lactoferrin in serum and milk during bovine subclinical mastitis. *J. Dairy Res.*, **88**(3): 321-325. DOI: 10.1017/S002202992100056X

Richardson, A.E., N. Hamilton, W. Davis, C. Brito and D. De León. 2011. Insulin-like growth factor-2 (IGF-2) activates estrogen receptor-α and -β via the IGF-1 and the insulin receptors in breast cancer cells. *Growth Factors*, **29**(2-3): 82-93. DOI: 10.3109/08977194.2011.565003

Ron, M., G. Israeli, E. Seroussi, J.I. Weller, J.P. Gregg, M. Shani and J.F. Medrano. 2007. Combining mouse mammary gland gene expression and comparative mapping for the identification of candidate genes for QTL of milk production traits in cattle. *BMC Genomics*, **8**(1): 183. DOI: 10.1186/1471-2164-8-183

Roth, S.M., M.A. Schrager, E.J. Metter, S.E. Riechman, J.L. Fleg, B.F. Hurley and R.E. Ferrell 2002. IGF2 genotype and obesity in men and women across the adult age span. *Int. J. Obesity*, **26**(4): 585-587. DOI: 10.1038/sj.ijo.0801927

Sadek, K., E. Saleh and M. Ayoub. 2017. Selective, reliable blood and milk bio-markers for diagnosing clinical and subclinical bovine mastitis. *Trop. Anim. Health Pro.*, **49**(2): 431-437. DOI: 10.1007/s11250-016-1190-7

Salman, M., M. Khaskheli, I.U. Haq, A.R. Talpur, A.P. Khuhro, M. Rauf, H. Hamid and A. Aziz. 2014. Comparative studies on nutritive quality of buffalo and cow milk. *International Journal of Research in Applied, Natural and Social Sciences*, **2**(12): 69-78.

Schalm, O.W., E.J. Carrol and N.C. Jain. 1971. *Bovine Mastitis*, Lea-Febiger, Phildelphia, USA. p. 94-124.

Servillo, L., N. D'Onofrio, G. Neglia, R. Casale, D. Cautela, M. Marrelli, A. Limone, G. Campanile and M.L. Balestrieri. 2018. Carnitine precursors and short-chain acylcarnitines in water buffalo milk. *Journal of Agricultural and Food Chemistry*, **66**: 8142-8149. DOI: 10.1021/acs.jafc.8b02963

Simões da Silva, T.M., A.C. Meirelles Piazentin, C.M. Nóbrega Mendonça, A. Converti, C.S. Bittencourt Bogsan, D. Mora and R.P. de Souza Oliveira. 2020. Buffalo milk increases viability and resistance of probiotic bacteria in dairy beverages under *in vitro* simulated gastrointestinal conditions. *J. Dairy Sci.*, **103**(9): 7890-7897. DOI: 10.3168/jds.2019-18078

Simões, P.B.A, M. Campbell, L. Viora, J. Gibbons, T.E. Geraghty, P.D. Eckersall and R.N. Zadoks. 2018. Pilot study into milk haptoglobin as an indicator of udder health in heifers after calving. *Res. Vet. Sci.*, **116**: 83-87. DOI: 10.1016/j.rvsc.2017.05.024

Spicer, L.J. and P.Y. Aad 2007. Insulin-like growth factor (IGF) 2 stimulates steroidogenesis and mitosis of bovine granulosa cells through the IGF1 receptor: role of follicle-stimulating hormone and IGF2 receptor. *Biol. Reprod.*, **77**(1): 18-27. DOI: 10.1095/biolreprod.106.058230

Szewczuk, M., M. Bajurna, S. Zych and W. Kruszyński. 2013. Association of insulin-like growth factor I gene polymorphisms (IGF1/TasI and IGF1/SnaBI) with the growth and subsequent milk yield of Polish Holstein-Friesian heifers. *Czech J. Anim. Sci.*, **58**(9): 404-411. DOI: 10.17221/6940-CJAS

Tatullo, M., B. Marrelli, C. Benincasa, E. Aiello, M. Amantea, S. Gentile, N. Leonardi, M.L. Balestrieri and G. Campanile. 2022. Potential impact of functional biomolecules-enriched foods on human health: A randomized controlled clinical trial. *Int. J. Med. Sci.*, **19**(3): 563-571. DOI: 10.7150/ijms.70435

Taylor, V.J., Z. Cheng, P.G. Pushpakumara, D.E. Beever and D.C. Wathes. 2004. Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. *Vet. Rec.*, **155**(19): 583-588. DOI: 10.1136/vr.155.19.583

Tripaldi, C. and G. Palocci. 2008. Milk composition and quality. *Bulletin of the International Dairy Federation*, **426**: 11-15.

Wang, C., X. Li, H. Dang, P. Liu, B.O. Zhang and F. Xu. 2019. Insulin-like growth factor 2 regulates the proliferation and differentiation of rat adipose-derived stromal cells via IGF-1R and IR. *Cytotherapy*, **21**(6): 619-630. DOI: 10.1016/j.jcyt.2018.11.010

Wilson, E.M. and P. Rotwein. 2006. Control of MyoD function during initiation of muscle differentiation by an autocrine signaling pathway activated by insulin-like growth factor-II. *Journal of Biological Chemistry*, **281**(40): 29962-29971. DOI: 10.1074/jbc.M605445200