Chemical Composition, in situ Ruminal Degradability, and Gas Production of Atriplex canesences, Salsola rigida and Aeluropus littoralis

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Introduction: Demands of agricultural products, particularly in developing countries, are increasing rapidly. Consequently, land and water resources are unable to sustain such demands. For decades, rangelands in Africa, West and Central Asia suffered from overgrazing and many areas are severely degraded. Range vegetation used to be the main feed resource for livestock particularly in the arid and semi-arid regions such as Iran. Soil and water salinity is another major constraint affecting dry land agriculture and following animal production as soil salinization is a major environmental problem in Iran. Climate change, human activities, reduced vegetation and a rising water table are contributing to expansion of saline soils worldwide. Halophytic plants can be regarded as feed resources for ruminants in Iran as identified 354 different species growing on highly saline sites. These plants are naturally grown or cultivated in salt affected lands such as in saline semi-deserts and could have great potentials particularly as sources of livestock fodders. However, wide intra- and inter-plant species variations in productivity, palatability, chemical composition, and nutritive value depend on seasonal changes, environmental conditions and management practices. Salt marsh plants, in particular chenopods forages, are relatively low in metabolizable energy and high in ash content. Data derived from many studies, on average values of chemical composition, crude fiber (3.4 to 14 percent) and dry matter digestibility (more than 60 percent) of most dominant halophytes in the Near East region have been determined. The aim of the study was to assess nutritive value of three most dominant halophytes in Semnan Province through determination of chemical compositions, in situ degradability and in vitro gas production techniques.

Materials and Methods: Nutritive value of Atriplex (A. canesences), Salsola (S. rigida) and Aeluropus (A. littoralis) were evaluated through determination of chemical compositions, in situ degradability and in vitro gas production techniques using three fistulated steers. The samples were collected at seed ripening period from Research Station in arid zone of Semnan Province and analyzed for chemical composition including crude protein (CP), ether extract (EE), ash, neutral detergent fiber (NDF), acetic acid, lignin (ADF), Ca, P, Na, K, Mg, Cl and S contents. Then, the samples were tested in an in vitro gas production method (96 h incubation) and batch rumen culture system (24 h incubation). The asymptotic gas production system (A) and lag time (L) parameters and also in vitro DM disappeared (IVDMD), organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acids (SCFA), N-NH3 content and pH were calculated. Subsequently, to determine the nutrients degradability of the plants, three fistulated steers were used. Approximately 5 g of each ground plant was weighed into Dacron bags with a pore size of 50 µm. Bags were incubated for 0, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96 and 120 h in the rumen. Zero-hour bags were only presoaked and washed. After rumen incubations, bags on were rinsed immediately with cold tap water, washed and dried at 60 ºC for 48 h to determine DM degradability. The residues from incubation time were used for determination of nutrient content (CP and NDF). Rumen degradation parameters of CP and NDF were estimated using the NLIN procedure of SAS.

Results and Discussion: All of these halophytic plants had high concentrations of Na, K, and Cl, and low levels of Ca, P and Mg. Moreover, the CP and ash contents of these plants were considerably high. It would be noticed that much of halophyte nitrogen is associated with non-protein compounds such as nitrates, glycine, betaine and proline. Other reports showed Atriplex shrubs contained higher level of ash. This result might be attributed to effects of salinity on cation accumulation as concentrations of some minerals in Atriplex spp. are higher than those recommended for ruminants and seem to reduce voluntary feed intake. There are variation between our found with results from other studies which is attributed to the difference in species, geographical regions, seasons and soil type. The asymptotic gas production system (A) and lag time (L) parameters were highest for Aeluropus. Also, OMD, ME, and SCFA parameters in Aeluropus were higher than Atriplex and Salsola. Furthermore, significant increase in gas produced after 96 h incubation was observed with Aeluropus in comparison to other plants. These results indicate that higher protein and lower NDF contents in Aeluropus may

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be attributed to different in gas production parameters. The amounts of OMD and ME were in line with results in other reports. However, the average fermentation rate (AFR) and pH were similar for halophyte plants. The rapidly degradable fractions of DM and CP of Salsola (28.42 and 55.68 %) were higher than those for Atriplex (23.24 and 48.7 %) and Aeluropus (21.50 and 51.28 %) which is associated with lower NDF and higher Ash contents in Salsola. On the other hand, degradability potential of DM and NDF of Aeluropus (52.42 and 75.82 %, respectively) were higher than those for Atriplex and Salsola. Moreover, Salsola showed the lowest effective degradability for NDF as measured at different outflow rates.

**Conclusion:** Overall halophyte plants were high in ash (Na, Cl and S) and CP but low in energy. Salsola plant may have better palatability because of lower NDF content and more soluble fraction (a) than to Aeluropus and Atriplex. The measured data in this study suggest that these halophytic plants can be regarded as feed resources for ruminants in the arid zones of Iran.

**Keywords:** Chemical composition, Gas production, Degradability, Halophytic plants
The Digestibility of Sugarcane Tops Silage by Rumen Bacteria or Microorganisms of Holstein Cows and Buffaloes of Khuzestan

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Introduction: Sugarcane (Saccharum officinarum) is widely cultivated in south west of Iran for production of sugar. After harvest, a lot of sugarcane tops remain on the farm that can be used to feed ruminants. The total digestible nutrients (TDN) and crude fiber of sugarcane top is about 72% and 39%, respectively. The digestibility of dry matter (54%) and crude protein (39%) of sugarcane top is low. Large amount of sugarcane top is wasted or burned annually. The sugarcane tops is important as forage for feeding of livestock. Preparation of sugarcane tops silage is a useful method to store and using it in whole seasons, prevent from wasting at the harvesting season and rich it using additives. Due to the low fermentable carbohydrate, and fibrous nature of sugarcane top, its durability of silage is short and quality of silage is inappropriate. Therefore, different methods can be used to enrich and enhance durability. The digestible protein of sugarcane top is low, so a proper nitrogen source is useful to improve the nutritional value of the sugarcane top. Using urea and molasses individually, is not suitable method for long term storage of sugarcane tops silage. The acids prevents from degradation of proteins in the silage, and decrease the pH rapidly. The bacteria have the most important role in digestion and degradation of starch, protein and plant cell wall. The aim of present experiment was to study the effect of sulfuric acid added to sugarcane top silage on digestion activity of rumen bacteria and whole rumen microorganisms, and followed by to compare the digestibility of sugarcane silage treated with urea + molasses and sulfuric acid between Holstein cattle and Khuzestan water buffalos.

Materials and Methods: The experimental treatments were ensiled sugarcane top by some additives included: 1) untreated (no additive) 2) urea (1%) + molasses (3%), 3) sulfuric acid (2.4% dry matter weight), 4) Both, urea+molasses and sulfuric acid. Rumen fluid was taken from buffalo and Holstein cow through rumen fistula.

Results and Discussion: The in vitro digestibility of dry matter (DM) of sugarcane top silage by total rumen microorganisms of cow was more than buffalo. Regardless of the type of animal, digestibility of DM by whole rumen microorganisms for urea+molasseses and acid, and digestibility neutral detergent fiber for acid was highest amount (P<0.05). By using two-stage digestion method and specific rumen bacteria culture medium method, digestibility of DM and neutral detergent fiber (NDF) was highest in treatment containing 2.4% acid sulfuric. The digestibility of DM by buffalo rumen bacteria was more than cow. Regardless of the type of animal in specific rumen bacteria culture medium, digestibility of DM and NDF by rumen bacteria for acid treatment was highest amount (P<0.05). Therefore acid had no negative effect on microorganisms, particularly bacteria. Even possibly because of sulfur in acid had a positive effect on their growing and thereupon digestion of nutrients. Therefore, sulfuric acid may be used for sugarcane top silage making.

Conclusion: The results of present experiment shown that using sulfuric acid not only had a negative effect on microorganisms, particularly bacteria, but also probably due to the presence of sulfur in its structure, had positive effect on their growing and thereupon digestion of nutrients. According to the results of this research, the digestibility of nutrients by whole rumen microorganisms of Holstein cow was more than river buffalo of Khuzestan. While, digestibility of nutrients by bacteria in the two-stage digestion and specific rumen bacteria culture medium methods, in buffalo was more than cattle. In general, in this study regardless of the type of animal, digestibility of DM NDF, in treatment containing sulfuric acid was more than other treatments. Probably at this level of sulfuric acid (2.4%), there was no decrease in the number of bacteria, or using 2.4% of

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commercial sulfuric acid had no negative effect on the bacteria or whole rumen microorganisms. Therefore, sulfuric acid can be exploited in the making of sugarcane silage.

**Keywords:** Silage, Specific rumen bacteria culture medium, Two stage digestion
Effect of Slow-release Bolus of Zinc, Selenium and Cobalt on Some Blood Metabolites and Performance of Male and Female Mehraban Lambs

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Introduction: Zinc (Zn) is cofactor of more than 300 enzymes and is needed for growth, DNA synthesis, immunity and other important cellular processes (Wood, 2000). Cobalt (Co) has been demonstrated to be an essential nutrient for ruminants and is required by ruminal microorganisms for the synthesis of vitamin B12 (McDowell, 2000). Selenium is an integral component of the enzyme glutathione peroxidase (GSH-Px) which neutralizes the effects of hydrogen peroxide and lipid hydroperoxide (Awadeh et al, 1998). Trace element deficiencies occur most frequently in the grazing animal where the use of extra food is limited. Supplementation of these grazing ruminants with trace elements can prove difficult. Trace elements can be supplemented in different ways. For example, the free-access methods (minerals, licks and blocks), but this suffers from variable intakes (Kendall, 1977). Oral drenches require frequent dosing in order to prevent deficiencies due to the short acting response of this type of supplement (Kendall et al., 2000). Injections require long term storage within the animal or frequent dosing. The controlled release bolus route should provide each animal with a consistent dose in line with its requirements sustained over a long period of time, such that one treatment of the animals should ensure adequate trace element cover for a number of months. The objective of our study was to determine the effects of slow-release bolus (Zn, Se and Co) on some blood metabolites and performance of male and female Mehraban lambs.

Materials and Methods: To conduct this experiment, 20 male and 20 female Mehraban lambs with average body weight of 30.37±1.71 kg grazing on the waste of agricultural land were used in a completely randomized design as 2×2 factorial experiment for 70 days. Treatments consisted of: 1) control male lambs not receiving slow-release bolus; 2) control female lambs not receiving slow-release bolus; 3) male lambs receiving a slow-release bolus; and 4) female lambs receiving a slow-release bolus. Final body weight, average daily gain, zinc, selenium, copper, iron, vitamin B12 concentrations of plasma, serum alkaline phosphatase activity, GSH-Px activity in whole blood and concentrations of serum T3, T4 and ratio of T3 to T4 were determined on days 35 and 70. Data collected for different parameters and determined in different days, were analyzed as repeated measures in a completely randomized design. The model used for analysis was: Yijkl = μ + Ai + Bj + ABij + Eaijl + Ebijkl

Where μ is overall mean, Ai: effect of treatment i, Bj: effect of day, ABij: interaction effect of treatment × day, Eaijl: error A, Ebijkl: error B. Duncan’s multiple range tests was used for comparison of means, considering P ≤ 0.05 as the significant level. The MIXED procedure of SAS (SAS Institute, 2004) was used for analysis of data.

Results and Discussion: The average final body weight, average daily gain, plasma concentrations of zinc and selenium, alkaline phosphatase and glutathione peroxidase activity, plasma concentrations of vitamin B12 in groups receiving slow-release bolus were significantly higher than the control group (P<0.05). Serum triiodothyronine (T3) amount increased (P<0.05), while serum thyroxine (T4) amount decreased (P<0.05). Serum ratio of T3/T4 in groups receiving slow-release bolus was significantly lower (P<0.05) than that of the control group. Glutathione peroxidase activity, the average final weight and average daily gain of male’s lambs were higher than those of female lambs (P<0.05). Copper and iron concentrations in plasma of male and female lambs were not affected by treatment (P>0.05). Similar to our findings, supplementation of 65 mg Zn/kg DM in the basal diet (15 mg Zn/kg DM) in female (Chhabra and Arora, 1985) and male (Chhabra and Arora, 1993) goats significantly increased serum Zn concentration. Kendall et al., (1997 and 2012) indicated that administration a slow-release bolus (Zn, Co and Se) to sheep was increased plasma Zn concentration in bloused animals than control group. Changes in the rate of plasma ALP tend to reflect changes in concentration of Zn in plasma among animals (Kirchgessner, 1980; Vergnes et al., 1990). It is reported that ALP activity increases at higher levels of Zn supplementation (Wan et al., 1993; Kraus et al., 1997; Nagalakshmi et al. 2009). It seems that the level of Zn was not sufficient for adequate ALP activity in lambs and the administration of a slow-release bolus (Zn, Co and Se) improved ALP activity. Selenium deficiency has been shown to reduce the activity of deiodinase in liver (Beckett et al. 1992). Similar to our findings in growing male calves (Arthur et al. 1988),

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heifers (Wichtel et al. 1996) and suckling calves, selenium supplementation increased plasma concentration of T_{3} and decreased T_{4}. Higher concentration of T_{3} in bolus receiving lambs in our study shows that deiodinase activity was improved by selenium supplementation. The only known animal requirement for cobalt is as a constituent of vitamin B_{12}, which has 4% cobalt in its chemical structure. This means that a cobalt deficiency is really a vitamin B_{12} deficiency (Berger, 2006). Microorganisms in the rumen are able to synthesize vitamin B_{12} needs of ruminants if the diet is adequate in cobalt. Normally, cobalt is not stored in the body in significant quantities. Zervas et al. (1988) indicated that administration a slow-release bolus (Cu, Co and Se) at 3 month prepartum to ewes increased the plasma concentrations of vitamin B_{12} until three months postpartum.

**Conclusion:** Overall results showed that slow-release bolus can meet the requirements of male and female lambs for the above mentioned trace elements and increase their performance. This result indicated that higher performance of bolus receiving group can be related to continuous release of Zn, Se and Co from the bolus.

**Keywords:** Lamb, Performance, Slow-release bolus, Trace element
Effect of Dietary Olive Leaves (Olea europaea L.) Extracts and/or \(\alpha\)-tocopheryl Acetate Supplementation on Performance and Intestinal Morphology of Chickens From 7 to 28 Days of Age

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Introduction: Antibiotic feed additives have long been used as growth promoters (10). However, consumers and health authorities increasingly dictate that the use of synthetic food additives should be phased out and, where possible, only natural products should be used. Hence, alternatives from natural sources with potential antioxidant activity are investigated (16). Olive leaves are a good source of several antioxidants including oleuropeoside compounds such as oleuropein and verbascoside, and flavonoid compounds such as luteolin, luteolin-7-glucoside, apigenin-7-glucoside, diosmetin, diosmetin-7-glucoside, rutin and catechin, and simple phenolic compounds such as tyrosol, hydroxytyrosol, vanillin, vanillic acid and caffeic acid. Oleuropein, the most prominent compound, may reach concentrations of 60 - 90 mg/g dried leaves (8). Most of these phenolic compounds have been shown to possess substantial antimicrobial and antioxidant activities in vitro. However, in vivo investigations on the antioxidant and antimicrobial activities of olive leaves as a dietary supplement to food producing animals have not been conducted (33). The present study was designed to evaluate the effects of dietary \(\alpha\)-tocopheryl acetate, olive leaf powder (OLP) and olive leaf extract (OLE) as feed additives on the growth performance and small intestinal morphology of male broilers.

Materials and Methods: The experimental protocol was approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran. The chickens (120 Ross 308 male birds) were placed at one day old in a single colony in a floor pen containing fresh wood shavings to a depth of 10 cm, and all were fed on a starter ration. At 7 d old, 6 replicate cages containing four chicks selected at random were assigned to each of 5 dietary treatments using a completely randomized design, giving a total of 30 experimental cages. The study was completed at 28 d of age. Dietary treatments included a negative control group (NC) fed a corn soybean meal based diet without feed additive supplementation, a positive control (PC) group fed a diet with 250 mg of \(\alpha\)-tocopheryl acetate/kg, a test group OLP fed with 10 g of olive leaf powder/kg, and two test groups remaining OLE fed with 120 or 240 mg of olive leaf extract/kg. The birds were provided with one hour of darkness following a period of 23 h light, from the start to the finish of the study. Each cage was provided with a single feed trough and nipple line, with three nipples per cage, to provide \textit{ad libitum} access to feed and water. On day 7, 14, 21 and 28, body weight and feed intake were measured after a 6-h fast to determine average daily gain, average daily feed intake, and feed conversion. On d 28, one bird per cage randomly selected and killed to determine small intestinal morphology and lymphoid organ weights. Sample sections (2.5 cm in length) were taken from the middle region of the jejunum. The tissues were stained for measurement of villus length, villus width, crypt depth and thickness of muscle layer. The General Linear Models of SAS (17) were used to analyze all the data.

Results and Discussion: Chicks fed with diet containing 240 mg of OLE/kg had the highest average daily gain. Dressing percentage and the percentage of breast muscle in the carcass were significantly higher for chicks fed diets supplemented with OLP and OLE compared with those fed NC diet. Similar to our results, Chinese herbal medicine supplementation enhanced BW gain of female broiler chicks at 7 to 21 day of age, when compared with the non-supplemented group. There were no significant differences in daily feed intake between the different treatments from 7 to 21 day of age (17). Also Christaki et al. (12) found that laying Japanese quails diet supplementation with olive leaves (10 g/kg or 20 g/kg) resulted in a tendency \((p = 0.054)\) for higher egg production percentage. The benefits of dietary olive leaves are possibly due to the presence of polyphenols and particularly oleuropein, the main active component in this material (12). The inclusion of OLP and/or OLE in diet significantly decreased the relative lengths of duodenum. Villus height, crypt depth, epithelial thickness and

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surface area for absorption villi were significantly increased in diets containing OLP and/or OLE compared with NC fed birds. Intestinal morphology can reveal some information on gut health. A shortening of the villus reflects villus atrophy and a decrease in surface area for nutrient absorption. The crypt can be regarded as the villus factory, and a large crypt indicates fast tissue turnover and a high demand for new tissue (21). The gastro protective potential of OLE is probably related to its ability to maintain the cell membrane integrity, by its ant lipid per oxidative activity that protects the gastric mucosa against oxidative damage and by its ability to strengthen the mucosal barrier, the first line of defense against exogenous damaging agents (14).

**Conclusion:** The use of OLP and/or OLE as feed additives in diet may affect chick performance and gut health. Although, it seems that further experiments are needed to elucidate the mechanisms underlying the enhanced growth of chickens by OLP and/or OLE.

**Keywords:** Broiler, Intestinal morphology, Olive leaf extract, Performance
Effects of Different Levels of Cinnamon Powder, Antibiotic and Probiotic on Performance, Blood Parameters and Immune System in Broiler Chickens

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Introduction: Dietary supplementation of medicinal plants as antibiotic replacement is a new approach in modern poultry farming systems. In poultry production, the main effects of medicinal plants are focused on the intestinal tract and its microbial flora. One of these medicinal plants is cinnamon that is known with scientific name *Cinnamomum verum* (syn. *Cinnamomum zeylanicum*). Cinnamon is native to Sri Lanka and southern India, but bark and leaf are widely used as a spice throughout the world. Cinnamaldehyde and eugenol are two very important terpenoids found in cinnamon, which are known as bioactive substances with potential health effects. They have intense antimicrobial, antifungal, and antioxidant properties. Cinnamon constituents possess antioxidant action and may prove beneficial against free radical damage to cell membranes.

Materials and Methods: This study was investigated the comparative effects of different levels of cinnamon powder (CNP), antibiotic (virginiamycin) and probiotic (primalac) on blood parameters and immune system in broiler chicks for 42 days using a completely randomized design. A total of 384, day-old male broiler chicks (Ross 308) were randomly assigned into 8 treatments with 4 replicates and 12 chicks each. The experimental treatments consisting of: Basal diet (BD), BD + 0.1% CNP, BD + 0.2% CNP, BD + 0.3% CNP, BD + primal, BD + 0.2% CNP + primal, BD + virginiamycin and BD + 0.2% CNP + virginiamycin. Virginiamycin was supplemented at 15 g/ton to the starter and 10 g/ton to the grower and finisher diets. Also primalac was added at 900 g/ton to the starter, 454 g/ton to the grower and 225 g/ton to the finisher diets. At the end of each phase, the average daily feed intake (ADFI), average daily weight gain (ADWG) and feed conversion ratio (FCR) for each group of birds were calculated and mortality was daily weighed, recorded and used to correct the FCR. At 42 d of age, one bird from each replicate with average pen weight was selected, blood samples were taken from wing vein and determined triglyceride, total cholesterol, ALT, AST, total protein and glucose in blood serum samples. SRBC (Sheep red blood cell) and CBH (Cutaneous basophil hypersensitivity) test were used to assessment of humoral and cellular immunity, respectively.

Results and Discussion: Apart from feed intake at starter period, dietary treatments had no significant effect on performance characteristics at starter, grower and finisher periods. The humoral immunity results showed that in first and second titer of SRBC test, IgG had lonely significant difference. In second titer of SRBC test, the response against antigen is faster and more powerful. Birds fed BD + 0.1% CNP had high second titer of IgG compared to treatments containing antibiotic and probiotic. Hypersensitivity test showed the treatment BD + 0.2% CNP + probiotic (primalac) increased toe membrane thickness at 24 hours after injection PHA-P solution. As cinnamon has potential broad antimicrobial and antioxidant activities, immune responses were expected to be elevated. It has been proved that herbal extracts increase anti-body titration against SRBC which herbal extracts stimulate immune response by increasing vitamin C and phagocytic cells activity. Immune responses using probiotics, medicines and plant extracts in the diet increases that were consistent with the results obtained in this study. Serum glucose and cholesterol levels were reduced with levels of 0.2% and 0.1% of CNP respectively. *Cinnamomum zeylanicum* inhibits the hepatic 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity, resulting in lower hepatic cholesterol content and suppresses lipid peroxidation via the enhancement of hepatic antioxidant enzyme activities. It is known that an unidentified factor present in cinnamon as methyl hydroxy chalcone polymers (MHCP). It was reported that MHCP presented in cinnamon increased insulin dependent glucose metabolism roughly 20 fold in vitro. Reduce serum glucose indirectly reduce the production of cholesterol, thereby reducing glucose to pyruvate production and reduced CoA which is a precursor of cholesterol.

Conclusion: This study showed that inclusion of CNP in broiler diets had not remarkable effects on performance, but the use of herbal additives such as cinnamon and probiotics as alternatives to antibiotics into diet may improve humoral and cellular immune responses and decreased serum glucose and cholesterol levels in

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the broiler chicks, thereby it can be useful meat quality of bird and human health.

**Keywords**: Blood parameters, Broiler chicks, Cinnamon powder, Immune system, Probiotic
Comparison of Regression and Artificial Neural Network Models in Predicting the Production Performance of Laying Hens

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Introduction: With using multiple linear regression (MLR), can simultaneously analyses several different variables, but to get the desirable results from the MLR, the samples must be much and accurate. Therefore, this method has high sensitivity and may cause errors in results. In addition, to use this method, the variable must have normal distribution and modification follow from a linear relationship. Artificial Neural Network (ANN) technique is used to solve a wide range of problems in science and engineering, particularly for some areas where the mathematical modeling methods fail. Nowadays, the ANNs are one of the most powerful modeling techniques to model complex nonlinear, multidimensional function relationships without any prior assumptions about the nature of the relationships. Artificial neural network models are different from mathematical modeling approaches in their ability to learn relationships between dependent and independent variables through the data itself rather than assuming the functional form of the relationships. A well trained ANN can be used as a predictive model for a specific application. The prediction by a well-trained ANN is normally faster than the mathematical models. Several authors have shown greater performances of ANN as compared to regression models. An ANN model can predict multiple dependent variables based on multiple independent variables, where a mathematical model is only able to predict one dependent variable at a time. Therefore, this study was designed to evaluate the prediction of production performance of laying hens using the neural networks and nonlinear regressions.

Materials and Methods: Review the four consecutive, information were obtained from a laying hen farm. Data mining methods include: three-layer perceptron neural network, four-layer perceptron neural network, radial basis function (RBF) neural network and multiply linear and nonlinear regression. In linear model, the variables of age flock, month of production, feed intake have been considered as the predictor variable and production (percent and egg mass production and feed conversion ratio) have been considered as the response variable. Three steps were taken to select an optimal ANN model. The first step was to determine the best number of hidden layers, number of neurons in each hidden layer, and activation function. The best models were selected on the basis of training and prediction accuracy. The second step was to work with the selected models to find the optimum epoch size. The third step was to find the optimum learning rate and momentum values. The evaluating method for selecting the optimal ANN was based on the minimization of deviations between predicted and measured values.

Results and Discussion: The aim of this study is to obtain an ANN model with minimum errors in training and testing. Nonlinear regression models were compared with Neural Network models. All the models are compared using the coefficient of determination (R2) and Mean Absolute Error (MAE). The results showed that the artificial neural networks compared the regression models and between different artificial neural networks the RBF model had better curve fitting for laying hen production performance indicators included; egg production (% / b / d), egg mass (g / b / d) and feed conversion ratio in front of age and this fact shows that even for spiral data artificial neural network works well. Therefore, we can use these models for complex situations. Conclusion: The obtained results revealed that the ANN model may efficiently be fitted into the laying hen production performance include percentage and egg mass and feed conversion ratio of hen flocks. Results showed that the method of radial basis function (RBF) neural networks acts better than other models in predicting the production performance of laying hens. So we can conclude RBF model performed better predict laying hen performance.

Keywords: Artificial neural networks, Laying hens, Production performance, Regression models

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Evaluation of Vermi-humus Supplementation in Japanese Quail’s Diets

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Introduction: The poultry industry has a crucial role in production of nutritious food for human consumption. Good quality feeds and rational nutrition are essential preconditions for obtain high producing healthy animals and economic prosperity in a flock. Antibiotics are worldwide used so far in animal husbandry to improve animal products, but nowadays the application of antibiotics as feed additives has been restricted. Therefore researchers have looked for new feed additives that are not harmful to human health. Previous studies related to humates have focused mainly on the growth of germinal tissue in seed. Humic acid based mixtures have the potential to be an alternative to antibiotic growth promoters in animal diets. The idea of using humates as feed additives in animal nutrition is new; in this research we investigated the effects of vermi-humus as an Iranian source of humic acid. Vermi-humus is final product of organic matter degradation by earth worms (Eisenia fetida). There are no information about nutritional effects of vermin-humus in poultry nutrition. The aim of the present study was to evaluate the effects of different levels of vermi-humus and virginiamycin on Japanese quail.

Materials and Methods: The treatments were considered as 6 levels of vermi-humus (0.2, 0.4, 0.6, 0.8, 1 and 2 %) and 0.15 % virginiamycin and a control diet in a randomized complete block design with 4 replicates and 10 birds in each cage. Therefore, 8 experimental dietary treatments were studied during 4 weeks. Water and feed were provided ad libitum, and lighting was continuous throughout the experimental period. The effects of these treatments were evaluated by general health indices such as growing performance, feed conversion ratio, viability, production efficiency factor, carcass percentage, relative organ weights, ileal microbial count, some serum biochemical indexes, and some meat quality parameters. The data were subjected to analysis using a General Linear Model procedure of SAS for the randomized complete block design. Differences between means were determined by Duncan’s multiple range test.

Results and Discussion: The lowest and the highest feed conversion ratio were related to 0.6 % vermi-humus and 2 % vermi-humus respectively. Dietary supplementation of different levels of vermi-humus did not influenced the viability percentage. There was no significant difference among birds that fed with supplemented diets with different levels of vermi-humus in compared with control group, in terms of Production efficiency factor. The lactic acid bacteria and streptococci counts in the 0.8 % vermi-humus were increased compared with other groups, moreover the Gram-negative bacteria count in this group showed a significantly reduction compared to other groups. The highest and the lowest concentration of serum cholesterol were observed in virginiamycin and 0.6 % vermi-humus, respectively. Serum triglyceride in the 0.8 % vermi-humus group showed a significantly decrease. The highest concentration of uric acid was observed in serum of birds that received virginiamycin. There was no significant difference among groups in terms of relative weights of heart, liver, proventriculus, and gizzard due to feeding vermin-humus. Also hemotocrit, hemoglobin, High density lipoprotein, total protein, albumin, phosphorus, percentage of tibia ash and meat ash were not affected by experimental treatments. The highest concentration of calcium was observed in serum of birds that fed with 0.6 % vermi-humus. The highest meat fat contents were related to of 0.6 % and 0.8 % vermi-humus, also the highest water holding capacity was observed in these groups and treatments with 1 % vermi-humus had a minimum malondialdehyde concentration. The positive effects of humic acid as a feed additive in this study were supported by the other studies, although some others do not demonstrated any beneficial effect of humate substances. Performance differences due to humate supplementation observed in the literature might result from the compositional differences among the commercially available humate products.

Conclusion: Overall, these results show that supplementing Japanese quail diets with vermi-humus at a level of 0.6-0.8 % can be used to improve quality of bird’s products along with beneficial effects to birds and humans. Therefore vermi-humus might be promising alternatives for antibiotic growth promoters as pressure to eliminate antibiotic growth promoters in animal feed increases. The vermi-humus offers a good alternative to improve

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quails production. These results show that vermi-humus at level of 0.6-0.8 \% can be used to improve quality of bird’s products along with beneficial effects to birds and humans.

**Keywords:** Blood parameters, Japanese quail, Performance, Vermi-humus
The Effects of Using Alfalfa-based Molt Diet Compared with Feed Deprivation on Blood Parameters Changes During Molt Period and Post-Molt Performance of Commercial Laying Hens

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Introduction: Induced molting is a process that stimulates natural molting events. When birds return to full feed, a new plumage develops and the birds resume egg production at a higher rate with better egg quality. Induced molting extends the productive life of commercial chicken flocks and results in substantial reduction in the number of chickens needed to produce the nation's egg supply. However, molting induced by feed withdrawal (FW) case discomfort and stress in hens. Stress causes a general deterioration of the well-being of chickens, usually involving a cascade of physiological adaptive responses that include changes in plasma levels of blood metabolites. The aim of the present study was determine the effects of alfalfa and feed deprivation on molt performance, some blood parameters changes during molt period and post-molt production of commercial laying hens.

Materials and Methods: This experiment was carried out through a completely randomized design with 108 of Hy-line W-36 laying hens at the 74 week of age in 3 treatments and 6 replicates (with 6 birds in each replicate). The using treatments for 12 days molt period were included: 1- control group fed with layer ration (FF), 2- feed withdrawal group (FW), 3- group fed with 90% alfalfa and 10% layer ration (A90). Blood sample was collected on day 0 (before any treatments began), 3, 6, 9 and 12 of molting period and serum concentration of triglyceride, cholesterol, glucose, calcium and uric acid were measured. At the end of this period, all hens return to the layer ration. Egg production was monitored for 12 weeks after the end of the molting period.

Results and Discussion: Results indicated that, triglyceride, calcium and uric acid levels were generally lower in molted hens compared with nonmolted hens during the molt period. On days 3 and 6 of molting, FF hens exhibited higher blood triglyceride levels than FW and A90 hens. In addition, the triglyceride levels measured in the A90 hens were significantly lower (P<0.05) than those of the FW birds. Similar results were observed on days 9 and 12 of molting period but no significant differences were found in triglyceride levels among hens molted by FW and A90 treatments. On days 3, 6, 9 and 12 of molting, serum uric acid from FF hens was significantly higher than other groups. On days 3, 6 and 12, FW hens had also higher levels of uric acid than A90 hens (P<0.05). Plasma uric acid in fasted or feed restricted hens was related to protein catabolism from either dietary sources or internal protein stores during the molt period. The FF hens generally had higher levels of calcium in the blood compared with FW and A90 hens. On days 3, 6, 9 and 12 of molting, the FF hens exhibited higher (P<0.05) concentrations of calcium in blood than did the other treatments, and FW hens were the lowest (P<0.05) except when compared with A90 hens on 12 day of molting. Calcium is mobilized from the bones and intestinal and transported to the reproductive tract for deposition in the eggshell gland, and decrease levels of eggshell gland and intestine calbindin occur during molting. Bone weight and density are known to decrease during a molt. Alfalfa contains 1.3% calcium and can be suitable source of this substance during molting period. The cholesterol levels of A90 and FW groups were significantly higher (P<0.05) than the FF non molted hens on days 6, 9 and 12 of molting period. But the differences were shown in cholesterol levels of hens in FW and A90 groups were not significant. Increases in serum cholesterol levels would be expected in molted hens, as the re-absorption of unovulated follicles during the process of molt increases the levels of circulating cholesterol fractions in the blood. In addition, failure to ovulate may also lead to an increase of an insoluble fraction of choleseterol, known as HDL<sub>4</sub>. The concentration of glucose was significantly lower in FW hens than other groups in early days of molting. On days 3 and 6 of molting, concentration of glucose were lower (P<0.05) in FW hens than in FF and A90 hens. Decreases in glucose levels would be expected in molted hens, as the reduction of feed intake during the molt process. Molted hens induced by alfalfa diet exhibited higher postmolt levels of egg production over a twelve week period than hens molted by feed withdrawal. The postmolt egg laid by hens

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molted by alfalfa were comparable quality to eggs from feed deprived hens.

**Conclusion:** In conclusion, the results of this experiment showed that the use of alfalfa for molt induction had favorable effects on some blood parameters during the molt period and improved the postmolt performance of hens.

**Keywords:** Alfalfa, Blood parameters, Forced molting, Laying hens, Performance
Up-regulation of Sox2 Gene in Cattle Fibroblast Cells using TALE-TFs

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Introduction
Cellular processes can be controlled by induction or repression of gene expression by introduction of exogenous genes and RNA interference (RNAi), respectively. However, the use of recently-developed specific DNA-binding transcription factors offer various advantages when compared to the exogenous gene introduction and RNAi for up or down regulation of gene expression. Sox2 gene encodes one of the most important transcription factors involved in the pluripotency state of mammalian cells. In this study the potential use of Transcription Activator-Like Effector-Transcription Factors (TALE-TFs) for endogenous up-regulation of Sox2 gene in cattle fibroblast cells were examined.

Materials and Methods
At first, a 320 base pair region of bovine Sox2 gene were sequenced and showed a 93.44% identity with its human orthologs. Based on the sequencing results, a previously reported Sox2-TALE-TF for human Sox2 gene was used for testing the functionality of TALE-TFs in cattle fibroblast cells. Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 50 µg/ml Penicillin and Streptomycin (Invitrogen), 1% non-essential amino acids, and 10% fetal bovine serum (FBS). For detecting the Sox2-TALE-TF activity the qRT-PCR technique and a fluorescent based reporter system under the control of minCMV promoter were used. For quantitative RT-PCR analysis of Sox2 mRNA levels, the cells were lysed, and total RNA isolated by using the RNeasy kit (Qiagen Inc.) and subsequently cleaned up with Turbo DNase (Ambion) according to the manufacturer’s instructions. Reverse transcription reaction was carried out using SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen) according to the manufacturer’s instructions. Real-time PCR analysis was performed on the 7900HT Fast Real-Time PCR system (Applied Biosystems) at standard reaction conditions using Power SYBR Green PCR Master Mix (Applied Biosystems). Briefly, after a 2 min denaturation at 95 °C, 40 cycles carried out at 95 °C for 15 s, 58 °C for 30 s and 72 °C for 30 s. Relative mRNA levels calculated with the \( \Delta\Delta C_T \) method were analyzed using SDS Version 2.4.1 software. Data presented are from an experiment with three replicates.

Results and Discussion
A set of known and as yet not understood molecular mechanisms regulate the expression of genes at (post-) transcription and (post-) translation stages in eukaryotic cells. It is generally agreed that there are three main routes of influence including; 1) combinatorial fashion of regulators and DNA 2) modulation through interaction of a control factor with the transcription machinery and 3) epigenetic modifications, are responsible for regulating gene transcription in various cell types. As a result of these mechanisms, the Sox2 gene is differentially expressed in a variety of embryonic cells and adult differentiated cell types. We choose the Sox2 gene for testing TALE-TF functionality in cattle fibroblast cells due to its well-known promoter structure as well as the lack of the evidence of methylation in the immediate regulatory regions. Our data shows that the Sox2 gene already has a basal level of expression in cattle fibroblast cells, indicating that Sox2 promoter has some activity in bovine fibroblast cells. As a proof of concept, we first tested activity of Sox2-TALE-TF in HEK293 cells. Florescent microscopy results showed that minCMV promoter has high level of activity in cattle fibroblast so it would not be considered as a suitable reporter of TALE-TF functionality. Analysis of qRT-PCR results for sox2 gene in cattle fibroblast, however, showed that Sox2 expression has been increased by 3.529 fold changes in transfected cells. Several mammalian genes have reportedly been targeted with TALE-TFs, however, fold changes in abundance of targeted gene transcripts rarely is above 5 times when individual TALE-TFs were tested. A number of factors affecting TALE-TFs activity have been reported, however, due to cell type specific chromatin state, multiple cis- and trans-acting regulatory elements and epigenetic modifications are involved in the regulation of gene expression, therefore it is very difficult to predict the efficacy of a TALE-TFs without experimental validation.

Conclusion
In summary current study indicated that TALE-TFs as artificial transcription factors have the ability to increase the endogenous expression of genes in cattle fibroblast cells.

Keywords: Cattle fibroblast, MinCMV promoter, Sox2 gene, TALE-TF

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The Effect of Reference Population Factors on the Quality of Genomic Selection in Holstein Cattle of Iran

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Introduction: Genomic selection refers to selection decisions based on genomic breeding values (GEBV). The GEBV are calculated as the sum of the effects of dense genetic markers across the entire genome, thereby potentially capturing all the quantitative trait loci (QTL) that contribute to variation in a trait. The QTL effects, inferred from markers, are first estimated in a large reference population with phenotypic information. In subsequent generations, only marker information is required to calculate GEBV. The success of genomic selection depends on the potential to predict genomic breeding values (GEBVs) with high accuracy. Genomic selection relies on relationships between individuals to accurately predict genetic value. Accuracy of genomic prediction is highly dependent on the size and type of the reference population (RP) used to estimate marker effects. For small populations, including information from other populations could improve this reliability. A usual strategy is to pool data from other populations.

Materials and Methods: Genome consists of 3 chromosomes each 100cM including 7500 markers with 0.04 cM space and 75 random distributed QTL were simulated. Genomic estimated breeding values of Iranian Holstein cattle were predicted using BayesB based on several reference dataset. Simulation was used to establish Iranian Holstein population and compare the accuracies of GEBVs under a range of different sizes and types of RP. The importance of information on relatives versus that of unrelated or more distantly related individuals on the estimation of genomic breeding values and effect of pooling data from other populations were also examined to construct the best RP for genomic selection in Iran.

Results and Discussion: The relationship between the animals in the test and reference data sets had high effect on the accuracy of genomic predictions. The increase of accuracy of GEBV by adding bulls in the RP was more than of adding dams indicating a direct relationship between the accuracy of predictions and the number of animals of reference population. whatever the relative relationship between the reference population was reduced by selecting animals, the accuracy is also reduced that in addition to showing the importance of the relationship between the two populations, suggest that Estimates should be repeated over time. The extent of linkage disequilibrium was similar in the Iranian and foreign Holstein populations and linkage disequilibrium between the two populations was very consistent and using the joint versus the Iranian reference dataset increased accuracy of genomic prediction.

Conclusion: The makeup of reference data sets is an important factor for the design of genomic evaluation systems to enable additional genetic gain from genomic selection at the lowest cost. An animal’s relationship to the reference data set is an important factor for the accuracy of genomic predictions. Animals that share a close relationship to the reference data set had the highest accuracy from genomic predictions. Our results suggest that the most accurate genomic predictions are achieved when data of dams and other population are combined by data of sires in RP.

Keywords: Genomic selection, Iranian Holstein, Reference population, Simulation

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Estimation of Genetic Parameters for Average Daily Gains and Kleiber Ratios in Guilan Province Sheep

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Introduction: The genetic characterization of local breeds of sheep is of paramount importance, not only for conservation purposes but also for the definition of breeding objectives and breeding programmes. When records are nonexistent and breeds are not well defined in the field, information could be obtained from research station information where pedigree and performance records have been kept. Early growth of animals is mostly dependent on the animal’s genetic merit for growth and maternal genetic and permanent environmental effects. Hence, both the direct and maternal genetic effects should be taken into account in a selection program to achieve optimum genetic progress. Studies on different sheep breeds have indicated that both direct and maternal genetic effects are essential for lamb growth. The efficiency of any sheep production enterprises can be improved through considering economically important traits such as litter size, body weight of lamb and quantity and quality of wool in the breeding objective. Growth is an economically important trait of farm animals which can be interpreted mathematically. It is a time (age) dependent change expressed in the weight or size of organ, composition of tissue/organ, size or number of cells and in live weight. Growth rate is related to rate of maturing and mature weight and these traits have been suggested to have association with lifetime productivity parameters in animals. Slow growth rate causes low market weight and has been identified as one of the limiting factors affecting the profitability of any production system. Selection of animals based on their predicted breeding values and accurate estimation of genetic parameters for economically important traits are required for designing optimal breeding strategies for farm animals. The Kleiber ratio has been suggested to be a useful indicator of growth efficiency and an indirect selection criterion for feed conversion under field conditions. The Guilan sheep is a fat-tailed breed of domestic sheep in Iran, numbering some 400,000 animals in the north of the country, and distributed in the northern and western parts of Guilan Province in the mountains between Assalem, Khalkhal, Oshkourat, and Deilaman. This breed can also be found in some areas of Guilan-Zanjandar border. Mean adult live weight in this breed is 35 kg (77 lbs) for rams and 31 kg (67 lbs) for ewes. The coat color for this breed is yellowish-white to pure white, but brown patches are found on the head, face and at the bottom of the legs. This breed is valued mainly due to its small size, meat, and ability to live in mountainous areas with rain-fed foothills and foothill steppes with 1300 mm (51 inches) rain. The objective of the present study was to estimate genetic parameters for average daily gain from birth to 3 (ADGₐ), 6-month of age (ADGₜ), average daily gain from 3 to 6-month of age (ADGₜ) and corresponding Kleiber ratios (KRₐ, KRₜ, KRₜ) in Guilan province sheep.

Material and Methods: Data and pedigree information used in this study were collected during years 1994 to 2011 by the Agriculture Organization of Guilan Province, Iran. The GLM procedure of SAS was used for determining the fixed effects which had significant influence on the under study traits. Level of significance for the inclusion of effects into the model of analysis was declared at P<0.05. Flock-year-season of lambing, lamb’s sex and dam’s age at lambing had significant effects on the under study traits. Genetic parameters were estimated with 6 different animal models using Restricted Maximum Likelihood (REML) procedure of Wombat program. The most suitable model was determined based on Akaike’s Information Criterion (AIC).

Results and Discussion: Based on the most appropriate fitted model, direct heritabilities of ADGₐ, ADGₜ, ADGₜ and corresponding Kleiber ratios were estimated to be 0.79, 0.18, 0.06, 0.25, 0.53 and 0.07, respectively. The lowest estimate of direct genetic correlation was between KRₐ-KRₜ (-0.48) and the highest estimate of direct genetic correlation was between ADGₜ-KRₗ (0.94). Estimated phenotypic correlations between average daily gains and Kleiber ratios were from -0.37 to 0.90 and those of environmental correlations were from -0.55 to 0.90. Estimations of genetic parameters for growth traits in this study showed that there are enough genetic diversity and as much as other breeds of sheep in Iran in Guilan province sheep and also genetic progress for these traits is possible by selection.

Keywords: Fat-tailed sheep, Genetic evaluation, Growth trait, Heritability

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Estimation the Trend and Genetic Parameters of Persistency of Holstein Cows

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Introduction: Lactation yield and persistency are two economic important traits in dairy cow. The production level of a dairy cow is determined by a joint effect of genetic and environmental factors. The main factors determining the total amount of milk production are milk yield at the peak of lactation and persistency as well as length of lactation period. Persistency are defined as a extent to which early lactation milk is preserved (22), or ability of cow to produce at a high level of milk during lactation period (8). Also, it’s defined as a decreasing rate of milk production after peak, that more milk Persistency means lower rate of milk yield (31). Persistency is as an economic important trait in dairy cattle because of the relationship between I tan other traits such as production, Reproduction, Health costs and Nutrition (28). In general, Selecting on milk production is caused weak reproductive performance (9 and 11), more Sensitivity to expose disease and increasing risk of culling due to it (11). One possible way to increase milk production without increasing the incidence of diseases and reproductive problems is to select for persistency as well as milk production. This strategy may be reduced stress of peak production and maintain high production after the peak. as a result of this selection, the lactation curve is flatter and persistency increases (17 and 24). So far, various functions and models are used to evaluate the appropriate daily milk production in dairy cows, Such that it can be pointed to exponential functions. The most famous application of this method was invented by Wood in 1967(15). Persistency would be estimated and evaluated by considering shape of lactation curve for each animal. The objective of present study was to estimate genetic parameters and trend of milk persistency for Holstein cows in Iran using Wood’s function.

Materials and Methods: Data consist of 2487378 test day of milk yield belonging to 336164 primiparous Holstein which calved from 1992 to 2012. At the first, persistency was estimated based on lactation curve parameters by the Wood’s function (32), mathematical form This function is as follows:

\[ Y_t = a e^{bt} e^{-at} \]

where, \( Y_t \): daily milk yield in \( t^{th} \) days in milk, \( t \): Days In Milk, \( e \): The exponential number, \( a \): a parameter representing yield at the beginning of Lactation, \( b \) and \( c \): Factors associated with the up ward and down ward slopes of the curve, respectively.

The Dim at peak production was defined as: \( T_{max} = \frac{b}{c} \), expected maximum yield (peak production) was calculated as: \( Y_{max} = a \frac{b}{c} e^{b} \), Persistency was calculated as: \( S = -(b+1) \ln(c) \).

The next step data were edited based on below conditions: animals were remove if they had two time calving only animals were evaluated that had at least 4 test day records, lactation period was limited from 5 to 305. parameters of lactation curve were estimated by nonlinear method in R software,then its variance components were estimated by REML method (AI-REML) under univariate animal model in wombat software.

The model for genetic evaluation of milk persistency was as follows:

\[ y_{ijk} = \text{MF}_i + \sum_{n=1}^{3} b_n f_{ij} c_{ij} + \sum_{n=1}^{3} b_n \text{DIM}_{ij} c_{ij} + HYS_j c_{ij} + a_k c_{ij} + e_{ijk} \]

\( y_{ijkm} \):Observation of \( k^{th} \)animal Persistency, \( i^{th} \)Milking frequency (MF), \( j^{th} \)-herd-year-season of calving, \( k^{th} \)age of first Calving and \( m^{th} \)Days In Milk (DIM), \( MF_i \):Fixed effect of Milking Frequency, \( AFC_{ij} \): Fixed effect of Age at First Calving as a covariate variable, \( \text{DIM}_{ij} \): Fixed effect of Days In Milk as a covariate variable, \( b_n \):regression coefficients, \( HYS_j \):\( j^{th} \)-herd-year-season of calving which was considered as a random effect, \( a_k \): Random additive genetic effect of\( k^{th} \)animal, \( e_{ijk} \): Random residual effect .

Results and Discussion: In general, amount of milk yield in beginning of the lactation was 14.19 kg (parameter of \( a \)) with an increasing steep (parameter of \( b \)) about 0.278 kg/day as well as 0.003 decreasing rate (parameter of \( c \)) in Holstein dairy cattle. time to peak (\( T_{max} \)) and milk yield at peak (\( Y_{max} \)) were 91 day and 33 kg, respectively. Heritability of persistency was low and estimated about 0.08 (Table 1). Results showed that

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phenotypic and genotypic trends were significant ($p < 0.05$) and estimated 0.022 and 0.01, respectively (Table 2).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Heritability ± SE</th>
<th>Additive genetic variance ± SE</th>
<th>Phenotypic variance ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>persistency</td>
<td>0.08 ± 0.004</td>
<td>0.03 ± 0.0014</td>
<td>0.37 ± 0.001</td>
</tr>
</tbody>
</table>

**Table 2- Phenotypic and genotypic trends of persistency**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Phenotypic trend ± SE</th>
<th>Genetic trend ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>persistency</td>
<td>0.022 ± 0.0006</td>
<td>0.01 ± 0.001</td>
</tr>
</tbody>
</table>

**Conclusion:** Low heritability of Persistency show that interested trait is affect by environmental effects compared with genetic effect. Therefore, for increasing Persistency of milk yield should be more pay attention to improve environmental factors such as herd health and animal nutrition. The results of the present study shows that the genetic and phenotypic trends milk persistency of Holstein cows were positive and favorable.

**Keywords:** Heritability, Milk production traits, Variance components