World Academy of Science, Engineering and Technology
International Journal of Animal and Veterinary Sciences
Vol:10, No:10, 2016

Effects of Cellular Insulin Receptor Stimulators with Alkaline Water on Performance, Plasma Cholesterol, Glucose, Triglyceride Levels and Hatchability in Breeding Japanese Quail

Rabia Göçmen, Gülşah Kanbur, Sinan Sefa Parlat

Open Science Index, Animal and Veterinary Sciences Vol:10, No:10, 2016 publications.waset.org/10005842/pdf

Abstract**—**Aim of this study is to determine the effects of cellular insulin receptor stimulators on performance, plasma glucose, high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol, triglyceride, triiodothyronine (T3) and thyroxine (T4) hormone levels, and incubation features in the breeding Japanese quails (*Coturnix japonica*). In the study, a total of 84 breeding quails was used, 6 weeks' age, 24 are male and 60, female. Rations used in experiment are 2900 kcal/kg metabolic energy and 20% crude protein. Water pH is calibrated to 7.45. Ration and water were administered ad-libitum to the animals. As metformin source, metformin-HCl was used and as chrome resource, chromium picolinate was used. Trial groups were formed as control group (basal ration), metformin group (basal ration, added metformin at the level of feed of 20 mg/kg), and chromium picolinate (basal ration, added feed of 1500 ppb Cr) group. When regarded to the results of performance at the end of experiment, it is seen that live weight gain, feed consumption, egg weight, feed conversion ratio (Feed consumption/ egg weight), and egg production were affected at the significant level ($p < 0.05$). When the results are evaluated in terms of incubation features, hatchability and hatchability of fertile egg ratio were not affected from the treatments. Fertility ratio was significantly affected by metformin and chromium picolinate treatments and fertility rose at the significant level compared to control group ($p < 0.05$). According to results of experiment, plasma glucose level was not affected by metformin and chromium picolinate treatments. Plasma, total cholesterol, HDL, LDL, and triglyceride levels were significantly affected from insulin receptor stimulators added to ration ($p < 0.05$). Hormone level of Plasma T3 and T4 were also affected at the significant level from insulin receptor stimulators added to ration ($p < 0.05$).

Keywords**—**Chromium picolinate, cholesterol, hormone, metformin, quail.

I. Introduction

PLASMA-glucose levels of poultries are almost two times higher than plasma glucose levels of most mammalian and poultry can protect high plasma glucose levels during rapid growth stage [1]. In poultries, glycogenesis occurs in liver and kidney in the rate of 70:30, respectively [2], [3]. Glycogenesis predominantly occurs in liver in mammals. In addition, liver glycogen depots of poultries are quite less than pigs and rats [4] and it rapidly runs out within 12 hours [5]. Poultries can protect high plasma glucose levels despite low glycogen stores; therefore, this suggests they have a unique gluconeogenesis mechanism.

Metformin-HCl is a compound used with priority in treatment of Type 2 Diabetics. Metformin reduces blood glucose without forming hypoglycemia [8], [9]. Type 2 diabetic patients have insulin resistance or they are obese patients who have hyperinsulinemia [6], [7]. High blood plasma glucose level, insulin resistance and obesity are symptoms of type 2 diabetics but for poultries, these are not normal. pH value of blood and intracellular fluid has to be between 7:35 to 7:45 for to be effective insulin and insulin receptors. When these limits are exceeded effectiveness of insulin and insulin receptors decrease.

Plasma insulin level of chickens is low and plasma glucose level of chicken is high [10] but the relationship between appetite and plasma glucose level of poultries could not be clearly understood. Reference [11] reported that, when metformin is administered to biguamide in acute dose, feed consumption, plasma glucose, insulin, and triglyceride decrease. These results also show that there is a relationship between feed consumption and plasma glucose in chickens. In the previous studies, it was seen that biguamide (metformin) reduced plasma glucose level in non-insulin dependent diabetics. The mechanism of this case is not fully known but in some conditions, it is considered that the decrease of hepatic glucose production may be the main benefit of metformin on diabetics [12], [13].

Chrome (Cr) is an essential trace element playing important role in carbohydrate, lipid, protein, and nucleic acid metabolism [14], [16]. Chrome has an insulin-like effect and it increases the permissibility of cell membrane to glucose and recently for chrome, the expression of glucose tolerance factor (GTF) is used. Chrome deficiency leads growth to slow; energy metabolism to be hindered and it causes even to diabetes mellitus and cardiovascular diseases [15]. American National Research Council [17] reported that trivalent chrome need was between 50 mcg/kg in adults. Cr contents of vegetable materials are lower than animal resources [18], [19]. Cr contents of vegetable materials are lower than animal

Gülşah Kanbur is with University of Selcuk, Institute of Science Department of Zootechnics, Konya, Turkey (corresponding author to provide phone: 0332 2233637; e-mail: gkanbur@ selcuk.edu.tr).

Rabia Göçmen and Sinan Sefa Parlat are with the University of Selcuk, Institute of Science Department of Zootechnics, and Konya, Turkey.

This study is supported by BAP office (Bilimsel araştırma Projeler ofisi – Projects office of scientific researchs) of Selcuk University (Effects of Cellular Insulin Receptor Stimulation on Performance, Some Blood Parameters and Hatching Properties in Breeder Japanese Quails-2016).

Open Science Index, Animal and Veterinary Sciences Vol:10, No:10, 2016 publications.waset.org/10005842/pdf

resources. Major part of poultry rations consists of vegetable feed materials; because of that, chrome deficiency is unavoidable situation in poultry. The number of studies about the ration Cr level effects is pretty few on the metabolism, health and performance in farm animals. Some study results [23], [24] showed that adding Cr to the practical rations of farm animals can be useful. Due to uncertainty about chrome effects on animal nutrition, American National Research Council (NRC) has prepared a compilation about chrome and its effects on laboratory and farm animals [20]. In all animal production areas for maximum and quality products to reveal genetic capacity of animals, it is necessary provide most compatible environment factors. One of the most important factor of in environmental factors is diet, it is more important especially in poultry because they dependent to ration to provide necessary nutrient components. Therefore, in the study, it was targeted to determine the effects of metformin and chromium picolinate on the performance, reproduction, and plasma metabolites.

II. Materials and Methods

A. Material

1. Animals

In this study, a total 84 of 6-week-old Japanese quails (24 male and 60 female) was used.

2. Feed Material

Some materials used in trial rations were bought from a commercial feed factory. Corn, soybean meal (SM), and sunflower meal (SFM), vegetable oil, marble powder, dicalcium phosphate (DCP), salt, premixes, and methionine were used as raw material. As a resource of metformin, metformin-HCL (20 mg metformin/kg) and as a resource of chrome, chromopicolinate (CrPic) (1500 ppb Cr/kg basal ration) were used.

B. Method

1. Experimental Design

In this experiment, rations and water were given to breeding quails as ad-libitum for 6 weeks. Water pH value was adjusted at 7.35 - 7.45. Also "16 hours light time and 8 hours dark time" lighting program was applied. A total of 84 6-week-old Japanese quails were distributed according to a completely randomised experimental design into 3 treatments, with 4 replicate cages of 2 male and 5 female quails each. In the experiment, groups were arranged as metformin group (20 mg metformin/kg), chromium picolinate group (1500 ppb Cr/kg basal ration) and control group (basal ration).

2. Performance Characteristics

Weight gain (WG) was calculated by weighing groups at the beginning and end of trail; feed consumption (FC) by subtracting the amount of feed remaining in feedbox from the amount of feed given; feed conversion ratio (FCR) by dividing the amount of feed consumed in a period by egg weight (EW) produced in the same period. Egg productivity (EP) was calculated according to egg periods. In the last week of experiment, 100 eggs collected from each group were placed incubation machine containing 37.5 °C heat and 55-60% humidity stored for 14 day. At the end of storage 14th day of the incubation, the eggs were transferred to exit machines containing 37.2 °C heat and 75% humidity and the exits were monitored. After the completion of the output, embryo deaths and infertility were determined by macroscopic analysis of eggs that did not produce chicks.

TABLE I
Raw Material and Nutritional Composition of Ration

Raw Materials	Breeding Quail Ration
Corn	55,00
SM	26,90
SFM	7,00
Vegetable oil	3,56
Limestone	5,52
DCP	1,30
Salt	0,35
Premiks1	0,25
DL-Methionine	0,12
Composition of calculate nutritional material	
Metabolic Energy, kcal/kg ME	2900
Protein, %	20
Calcium, %	2,5
Phosphor, %	0,35
Methionine, %	0,45
Methionine + Cysteine	0,8
Lysine, %	1,05

Provided/kg of diet; Mn: 60 mg; Fe: 30 mg; Zn: 50 mg; Cu: 5 mg; 1, 1.1 mg; Se: 0.1 mg, Vitamin A, 8.800 IU; Vitamin D3, 2.200 IU; Vitamin E, 11 mg; Nicotine acid, 44 mg; Cal-D-Pan, 8.8 mg; Riboflavin 4.4 mg; Thiamin 2.5 mg; Vitamin B12, 6.6 mg; Folic acid, 1 mg; D-Biotin, 0.11 mg; Choline: 220 mg.

3. Biochemical Analyses

At the end of experiment, blood samples were taken from 4 animals, randomly selected from each repeat, and serum was obtained by centrifuging for 5 minutes in 3500 rpm. In the serums obtained, the values of glucose, HDL, LDL, total cholesterol, and triglyceride levels were determined by chromatic analysis method in dimension device.T3 and T4 hormone levels of plasma were determined by chemiluminescence method in ADVIA centaur XP device.

4. Statistical Analyses

A statistical analysis of the results was performed using the ANOVA of Minitab program according to the following general model

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

μ: General average; **α_i:** Effect of treatments examined; **eij:** Effect of error and Duncan's multiple range tests were applied to the separate means [21].

III. Result and Discussion

In breeding quails, the results and standard errors of adding metformin and chromium picolinate to rations are presented in Table II.

TABLE II
PERFORMANCE CHARACTERISTICS

Experimental groups	Control	Metformin	CrPic	P value
WG	21,75±2,78[b]	34,25±2,39[a]	34,25±2,25[a]	0,009
FC	26,86±0,65[b]	29,42±0,31[a]	27,27±0,21[b]	0,005
EW	9,49±0,12[b]	7,17±0,16[c]	10,48±0,12[a]	0,000
FCR	2,84±0,10[b]	4,14±0,12[a]	2,67±0,03b	0,000
EP	70,19±2,81[b]	80,25±2,09[a]	76,93±0,97[a]b	0,022

a, b: Means with different minuscule in the same row are significantly different at $P < 0.05$.

TABLE III
INCUBATION FEATURES

Experimental groups	HR (%)	FR (%)	HOF (%)
Control	77,08±3,99	83,33±4,81[b]	93,18±6,29
Metformin	87,50±2,41	97,92±2,08[a]	89,39±2,03
Chromium picolinate	77,08±3,99	95,83±2,41[ab]	80,49±4,15
P value	0,108	0,026	0,179

a, b: Means with different minuscule in the same column are significantly different at $P < 0.05$.

WG was found significantly high in both metformin and chrome picolinate groups compared to control group ($p < 0.05$). While FC, with the addition of metformin, was found significantly high compared to control group, it was identified that chromium picolinate group was also at the same significance level with control group ($p < 0.05$). EW was found high at the significant level in Chromium picolinate group, compared to control and metformin groups and the lowest EW was identified in metformin group ($p < 0.05$). It was found that FCR was significantly high compared to both chromium picolinate and metformin groups ($p < 0.05$). EP was found high at the significant level in both Chromium picolinate and metformin group compared to control group and the highest EP was identified in metformin group ($p < 0.05$).

Studies about effects of metformin in poultries on performance are limited; however, [22] reported that by addition 5 and 10 g/kg diet levels of metformin to ration in broiler reduced feed consumption. This research is not compatible our study according to feed consumption results considered with doses and animal species differences, at the same time in our study, with metformin addition to feed, improvement of FCR but rise of WG and EP point out that this compound has positive effects on performance in poultries. Reference [23] showed that 500 ppb level in ration of chrome obtained from chrome yeast in broiler improved WG and FCR significantly. Reference [24] found that the addition of Cr at the levels of 1600-3200 µg/kg increased feed consumption at the significant level, and addition of at the level of 1600 µg/kg improved increase of live weight. In our study, in parallel with the studies above mentioned, addition of chromium picolinate to ration while WG rose significantly, feed consumption was identified at the same level as control group; however, EW and EP were found high compared to control group.

In breeding quails, the results of incubation features related to addition of metformin and chrome picolinate and standard errors are presented in Table III.

When regarded to incubation features, it is seen that HR and HOF are not significantly affected from addition of metformin and Chromium picolinate to rations ($p > 0.05$). However, although it is not statistically significant, the highest value of HR was identified in the group added metformin. With addition of metformin and chromium picolinate, FR was affected at the significant level and, also in both groups, higher results were obtained compared to control group ($p < 0.05$).

Reference [25] reported that sperm quality, activity, and density increased and sperm deformation decreased of cocks by adding chrome yeast in ration. These changes occurred in sperm will likely affect fertility and thus incubation performance. These results are compatible with our existing study and it can be said that chromium picolinate addition in doses fit to ration may affect incubation performance in the positive direction. Although there is not any study in which the effects of metformin on reproduction performance in poultries or the other farm animals were reported, some studies showing its effect on reproduction in humans were met. Reference [26] reported that ovulation activity improved with eleven weeks metformin treatment 70% of patients in adolescent girls who are not obese. This result points out that reproduction pathology depends on metabolism and it may improve with the metabolic changes. In light of these data, in poultry or farm animals by determine suitable doses for both compounds production problems can solve

In breeding quails, the blood parameters and standard error of adding metformin and chromium picolinate to rations are presented in Table IV.

TABLE IV
BLOOD PARAMETERS

Experimental groups		Control	Metformin	CrPic	P value
Glucose	**(mg/dL)**	327,8±5,77	317,5±4,58	337,5±12,43	0,287
HDL	**(mg/dL)**	14,50±1,32[b]	15,75±0,48[ab]	20,50±1,76[a]	0,023
LDL	**(mg/dL)**	26,25±2,28[a]	19,50±0,65[b]	16,50±1,26[b]	0,000
Total Cholesterol	**(mg/dL)**	113,3±3,55[b]	129,0±3,49[a]	108,3±3,57[b]	0,006
Triglyceride	**(mg/dL)**	976,0±27,15[a]	784,8±31,41[b]	741,3±20,76[b]	0,000
T_3	**(ng/dL)**	0,3563±0,006[a]	0,2663±0,015[b]	0,1655±0,007[c]	0,000
T_4	**(ng/dL)**	0,688±0,041[b]	0,933±0,021[a]	0,895±0,074[ab]	0,016

a, b: Means with different minuscule in the same row are significantly different at $P < 0.05$.

With regard to blood parameters and hormone results, it is seen that adding metformin and chromium picolinate to ration did not affect the plasma glucose level significantly ($p > 0.05$). The lowest plasma glucose level was found in metformin group ($p < 0.05$). Plasma HDL level was found significantly high and plasma LDL level was found significantly low in both groups when compared the control group ($p < 0.05$). It was found that in metformin group, total cholesterol is

Open Science Index, Animal and Veterinary Sciences Vol:10, No:10, 2016 publications.waset.org/10005842/pdf

Open Science Index, Animal and Veterinary Sciences Vol:10, No:10, 2016 publications.waset.org/10005842/pdf

significantly higher than that in control and Chromium picolinate groups and that addition of metformin and Chromium picolinate significantly reduced plasma triglyceride level compared to control group ($p < 0.05$).

End of experiment was determined that both compounds effected on T_3 and T_4 hormones and we found that T_3 hormone level decreased while T_4 hormone level increased with the effect of these compounds ($p < 0,05$).

Plasma insulin hormone levels was found in all groups under 2 μIU/mL and insignificant therefore it isnot given in Table IV, in terms of serum glucose levels found no significant difference between groups and it was not expected result. Some researchers reported that serum glucose decreased with chrome [24], [27], [28].

Reference [29] reported that designated plasma glucose level which stable during 24 hours fell after metformin treatment. Reference [22] reported that plasma glucose level was not affected from the addition of metformin. The results of study mentioned are in compatible with present study.

Reference [30] reported that chromium picolinate, biotin or their combinations of both (except of the lowest dose of chromium picolinate) increase serum HDL level significantly.

Reference [31] reported that adding Cr to ration reduced the serum LDL level in Japan quails. As a result of the study, in which the effect of adding Morina liver fat and Chromium picolinate on hens is studied, it was reported that also in both groups, the content of serum triglyceride and cholesterol ($P < 0.05$-0.01) and egg yolk cholesterol are lower compared to control group ($P < 0.01$); in Chromium picolinate group, the value HDL was high; and the values of LDL and VLDL were lower ($P < 0.05$)

Reference [22] reported that addition of metformin did not affect plasma cholesterol levels of chicken. Reference [32] reported with addition of metformin, the values of serum triglyceride and cholesterol decreased in the increasing dose.

Present study is compatible with the studies concerned. It is considered that the possible reasons for incompatibilities are the doses used. Compared to control group, in metformin and Chromium picolinate groups, while T_3 hormone level decreased, T_4 hormone level increased. Especially, in farm animals, a literature information, according to which the effects of addition of Chromium picolinate and metformin to ration are studied, could not be unfortunately reached. However our research's results showed these compounds effective on T3 and T4 hormones level due to we can say that need more research about metformin and chrome picolinate compounds as known effects on metabolism.

IV. Conclusion

As a conclusion, metformin and Cr Pic are the effective compounds on metabolism which were added to breeding Japanese quail rations. However, although one of the primary effects expected in the experiment was serum glucose fall, it was not observed. It is likely that; this result is due to the sufficiency in doses used. As a conclusion of this study, it is considered that using both compounds in poultries in suitable doses and especially together with water having high pH will improve performance.

Also results were shown positive effect on fertility of these compounds and thus on reproductive performance. At the end of the experiment; it was determined that HDL level (called good cholesterol) increased, LDL (called bad cholesterol) and triglyceride levels reduced. As a result, we can say that these components may use to produce qualified products which has low cholesterol and triglyceride levels

References

[1] N. Sarkar, "Gluconeogenesis and the factors that control the process in chickens", *Life sciences*, 1971, pp.(10) 293-300.

[2] M. Watford, Y. Hod, Y.B. Chiao, M.F. Utter, R.W. Hanson," The unique role of the kidney in gluconeogenesis in the chicken. The significance of a cytosolic form of phosphoenolpyruvate carboxykinase", *Journal of Biological Chemistry*, 1981, pp. (256) 10023-10027.

[3] M. Watford, "Hormonal and nutritional regulation of phosphoenolpyruvate carboxykinase mRNA levels in chicken kidney", *The Journal of nutrition*, 1989, pp. (119) 319-322.

[4] P.S. Belo, D.R. Romsos, G.A. Leveille, Blood metabolites and glucose metabolism in the fed and fasted chicken, *J. Nutr*, 106 (1976) 135-131.

[5] T.F. Davison, D.R. Langslow, "Changes in plasma glucose and liver glycogen following the administration of gluconeogenic precursors to the starving fowl", *Comparative Biochemistry and Physiology Part A: Physiology*, 1975, pp. (52) 645-649.

[6] G.A. Bray, "Drug treatment of obesity", *Reviews in endocrine and metabolic disorders*, 2001, pp.(2) 403-418.

[7] K. Cusi, R.A. DeFronzo, "Metformin: a review of its metabolic effects", *Diabetes Rev.,* 1998, pp.(6) 89–131.

[8] A. Lee, J.E. Morley, "Metformin Decreases Food Consumption and Induces Weight Loss in Subjects with Obesity with Type II Non-Insulin-Dependent Diabetes", *Obesity research*, 1998, pp. (6) 47-53.

[9] J. Rouru, R. Huupponen, U. Pesonen, M. Koulu, "Subchronic treatment with metformin produces anorectic effect and reduces hyperinsulinemia in genetically obese Zucker rats", *Life sciences*, 1992, pp.(50) 1813-1820.

[10] J. McMurtry, R. Rosebrough, N. Steele," A homologous radioimmunoassay for chicken insulin", *Poultry science*, 1983, pp.(62) 697-701.

[11] C. Ashwell, J. McMurtry, "Hypoglycemia and reduced feed intake in broiler chickens treated with metformin", *Poultry science*, 2003, pp. (82) 106-110.

[12] M. Otto, J. Breinholt, N. Westergaard, "Metformin inhibits glycogen synthesis and gluconeogenesis in cultured rat hepatocytes", *Diabetes, Obesity and Metabolism*, 2003, pp. (5) 189-194.

[13] A. Kiersztan, A. Modzelewska, R. Jarzyna, E. Jagielska, J. Bryła, "Inhibition of gluconeogenesis by vanadium and metformin in kidney-cortex tubules isolated from control and diabetic rabbits", *Biochemical pharmacology*, 2002, pp. (63) 1371-1382.

[14] S. Okada, H. Tsukada, H. Ohba, "Enhancement of nucleolar RNA synthesis by chromium (III) in regenerating rat liver", *Journal of inorganic biochemistry*, 1984, pp. (21) 113-124.

[15] T.G. Page," Chromium, tryptophan, and picolinate in diets for pigs and poultry" *A. dissertation Lousiana State University U. S. A*, 1991.

[16] N.C. Steele, R.W. Rosebrough, "Effect of trivalent chromium on hepatic lipogenesis by the turkey poult", *Poultry science*, 1981, pp. (60) 617-622.

[17] NRC, Nutrient Requirements of Poultry, in: N.R. Council (Ed.), *National Academy Press Washington* DC., 1989.

[18] R.S. Gibson, "Assessment of trace element status in humans, Progress in" *food & nutrition science*, 1988, pp. (13) 67-111.

[19] H.A. Schroeder, "Losses of vitamins and trace minerals resulting from processing and preservation of foods", *American Journal of Clinical Nutrition*, 197, pp. (24) 562-573.

[20] NRC, Nutrient Requirements of Poultry, in: N.R. Council (Ed.) *National Research Council*, National Academy Press Washington DC., 1994.

[21] O. Düzgüneş, T. Kesici, F. Gürbüz, "İstatistik Metotları" I., *A.Ü. Ziraat Fakültesi Yayınları* 1984.

[22] R.W. Rosebrough, C.M. Ashwell, "Dietary metformin effects on *in vitro* and *in vivo* metabolism in the chicken", *Nutrition research*, 2005, pp. (25) 491-497.
[23] B. Kroliczewska, W. Zawadzki, Z. Dobrzanski, A. Kaczmarek-Oliwa, "Changes in selected serum parameters of broiler chicken fed supplemental chromium", *J Anim Physiol*, 2004, pp. (88) 393-400.
[24] T. Lien, Y. Horng, K. Yang, "Performance, serum characteristics, carcase traits and lipid metabolism of broilers as affected by supplement of chromium picolinate", *British poultry science,* 1999, pp. (40) 357-363.
[25] C. Yufang, "Studies on effect of yeast chrome on sperm quality of breeder cocks under heat stress and its action mechanism", *Journal of Anhui Agricultural Sciences*, 2007, pp. (35) 20-29.
[26] L. Ibáñez, N. Potau, A. Ferrer, F. Rodriguez-Hierro, M.V. Marcos, F. de Zegher, "Anovulation in eumenorrheic, nonobese adolescent girls born small for gestational age: insulin sensitization induces ovulation, increases lean body mass, and reduces abdominal fat excess, dyslipidemia, and subclinical hyperandrogenism", *The Journal of Clinical Endocrinology & Metabolism,* 2000, pp. (87) 5702-5705.
[27] M.A. Cupo, W.E. Donaldson, "Chromium and vanadium effects on glucose metabolism and lipid synthesis in the chick", *Poultry science,* 1987, pp.(66) 120-126.
[28] K. Şahin, O. Küçük, N. Şahin, O. Ozbey, "Effects of dietary chromium picolinate supplementation on egg production, egg quality and serum concentrations of insulin, corticosterone, and some metabolites of Japanese quails", *Nutrition Research*, 2001, pp.(21) 1315-1321.
[29] T. Sato, A. Toyoshima, T. Hiraki, Y. Ohta, K. Katayama, T. Arai, H. Tazaki, "Effects of metformin on plasma concentrations of glucose and mannose, G6Pase and PEPCK activity, and mRNA expression in the liver and kidney of chickens", *British poultry science*, 201, pp. (52) 273-277.
[30] J.R. Komorowski, H.J. Dela, W.T. Cefalu, "LA-cp rats show improved lipid profiles in response to diets containing chromium picolinate and biotin" *Meeting of the Society for the Study of Ingestive Behavior*, Philadelphia, 2001.
[31] F. Uyanik, Ş. Kaya, A.H. Kolsuz, M. Eren, N. Şahin," The effect of chromium supplementation on egg production, egg quality and some serum parameters in laying hens", *Turkish Journal of Veterinary and Animal Sciences*, 2002, pp. (26) 379-387.
[32] W.-L. Chen, H.-W. Wei, W.-Z. Chiu, C.-H. Kang, T.-H. Lin, C.-C. Hung, M.-C. Chen, M.-S. Shieh, C.-C. Lee, H.-M. Lee, 'Metformin regulates hepatic lipid metabolism through activating AMP-activated protein kinase and inducing ATGL in laying hens", *European journal of pharmacology*, 2011, pp.(671) 107-112.

Open Science Index, Animal and Veterinary Sciences Vol:10, No:10, 2016 publications.waset.org/10005842/pdf