



Original Research

Effect of Graded Levels of Shatavari Root Meal on the Body Weight, Feed Conversion Ratio, Biochemical Attributes and Immune Competence Traits of Coloured Chicken

Binay Kumar Yadav, Pankaj Kumar Shukla, Amitav Bhattacharyya*, Debashis Roy¹, Aditya Sharma and Rajneesh Sirohi²

Department of Poultry Science, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, INDIA

¹Department of Animal Nutrition

²Department of Livestock Production and Management

*Corresponding author: amitav16@rediffmail.com

Rec. Date:	Sep 22, 2017 11:16
Accept Date:	May 12, 2018 12:31
DOI	10.5455/ijlr.20170922111622

Abstract

Two hundred and ten straight run, day old coloured chicken (Chabro) were randomly distributed into seven dietary treatments T1- basal diet (broiler starter diet till 4 weeks, broiler finisher diet till eight weeks), T2- T1+0.25% Shatavari root meal (SRM), T3- T1+0.5% SRM, T4- T1+0.75% SRM, T5- T1+1% SRM, T6- T1+1.25% SRM and T7- T1+1.5% SRM, having three replicates each with ten coloured chicken. T2 birds had a significantly better ($P<0.05$) FCR at 2nd week as compared to T1, T6 and T7. T3 coloured birds had significantly better ($P<0.01$) cell mediated immune response than T1, T4, T5 and T7 at 8 weeks of age. T1 and T2 had significantly higher ($P<0.01$) plasma cholesterol than the other treatment groups. Hence, dietary supplementation of Shatavari meal @ 0.5% and above reduced plasma cholesterol in coloured chicken. Further, dietary supplementation of Shatavari root meal @ 0.5% may improve growth performance and immunity in coloured chicken.

Key words: Body Weight, Coloured Chicken, Immunity, Shatavari

How to cite: Yadav, B., Shukla, P., Bhattacharyya, A., Roy, D., Sharma, A., & Sirohi, R. (2018). Effect of Graded Levels of Shatavari Root Meal on the Body Weight, Feed Conversion Ratio, Biochemical Attributes and Immune Competence Traits of Coloured Chicken. International Journal of Livestock Research, 8(10), 115-123. doi: 10.5455/ijlr.20170922111622

Introduction

Poultry production has witnessed a phenomenal growth from backyard farming to scientific poultry farming involving commercial egg and meat production in India. Though Indian poultry industry recorded phenomenal growth, it has been plagued by a series of problems due to high ambient temperature in the tropics accompanied by high relative humidity. Backyard poultry has been a means for earning livelihood



for the economically distressed farmers due to its promising results in productivity from the improved backyard birds. Thus, backyard poultry farming is being promoted for sustainability of poultry production and up liftment of socio-economic condition of Indian farmers. Chabro is a breed, developed by Central Poultry Development Organization (CPDO) especially for the farmers of our country as it is comparatively more adaptive to climatic fluctuations and variable zoo sanitary conditions of the farm as compared to their broiler counterparts.

In the recent past, efforts have been made to counteract the adverse effects of various levels of stress and augment the production potential in broilers by using herbs possessing therapeutic potential. Hence, studies have been undertaken to assess the effect of dietary supplementation of Shatavari (*Asparagus racemosus*) root powder to augment the growth of broilers (Sharma *et al.*, 1986; Mane *et al.*, 2012). Recently, studies have also been undertaken to assess the effect of Shatavari root meal on the immunity, blood biochemical attributes and carcass quality characteristics of broilers (Kant *et al.*, 2014; Dahale *et al.*, 2014). However, detail studies are necessary to assess the effect of Shatavari root meal at various levels on the productive performance and immunity of coloured chicken. Hence, the present study was conducted to determine the effect of Shatavari root meal at graded levels on the body weight, feed conversion ratio, biochemical attributes and immune competence traits of coloured chicken.

Materials and Methods

Raw Shatavari root was obtained from the Instructional Livestock Farm Complex Campus of the University. Fresh root was sun dried in a clean, dust free environment and ground to obtain fine powder. The powder formed was packed in an airtight container.

Birds and Feed

Two hundred and ten straight run, day old Chabro chicks were divided into seven treatment groups comprising three replicates of ten chicks each. The feed was offered *ad lib* in different experimental groups. The birds of the control group, T1 were fed basal or control diet (BIS 2007; broiler starter diet till 4 weeks and there after broiler finisher diet till eight weeks), T2- T1 + supplementation of 0.25% Shatavari root meal, T3- T1+ supplementation of 0.5% Shatavari root meal, T4- T1+ supplementation of 0.75% Shatavari root meal, T5- T1+ supplementation of 1% Shatavari root meal, T6- T1+ supplementation of 1.25% Shatavari root meal and T7- T1+ supplementation of 1.5% Shatavari root meal. The birds were housed in deep litter system. Water was offered *ad lib*.

Body Weight and Feed Conversion Ratio

Weekly body weight and group feed consumption was recorded. Thereafter, weekly feed conversion ratio was calculated at the end of the experiment.

Blood Biochemical Attributes

Blood was collected from six chicken of each group at the end of the biological experiment from the wing vein with the help of heparinized syringes and poured into sterile tubes. The blood samples were centrifuged for the 10-15 min at 2500 rpm. Plasma was separated and stored in refrigerator (-20°C) until analyzed. Plasma cholesterol, HDL cholesterol, protein, uric acid, SGPT, SGOT, alkaline phosphatase were determined by using commercial kits of Span Cogent Diagnostics Product, India, according to the manufacturer's instructions.

Immunocompetence Traits

The general innate immune-competence status of chabro birds was assayed by measuring two important immunocompetence traits as antibody response to SRBC and cell mediated immune response to PHA-P at 8 week of age.

Antibody Response to Sheep Red Blood Cells (SRBC)

The microtitre plate haemagglutination procedure as described by (Siegel and Gross 1980) with slight modifications was followed to measure total HA antibody titres in chabro birds on day zero and day 5 post injection. The procedure followed is described below.

Preparation of Sheep Red Blood Cells (SRBC) Suspension

Blood from jugular vein was collected from healthy sheep in Alsever's solution. The red blood cells were washed thrice in PBS (phosphate buffer saline, pH 7.2). Finally 1% suspension of SRBC in PBS (V/V) was prepared.

Immunization and Harvesting of Immune Serum

1 ml of 1% (V/V) of SRBC suspension was injected to 6 birds of each treatment group. About 3 ml of blood on 0 and 5th day post immunization (dpi) were collected from jugular vein. The blood was endorsed to clot in an incubator having temperature of 37°C for 1 hour. The blood was endorsed to retract after detaching it from sides of its container and left at 4°C. Centrifugation of blood was carried out at 2000rpm for 5-10 minutes as it facilitated rapid collection of serum. The antibody titer was determined by HA methods (Vander Zijpp 1983; Siegel and Gross, 1980). Antibodies were determined by means of a mercaptoethanol (ME) HA test as per the method described by Martin *et al.* (1989) with slight modification.

***In vivo* Cell Mediated Immune Response**

The cellular immune response was assessed by cutaneous basophilic hypersensitivity test *in vivo* by using PHA-P (Phytohaemagglutinin, lectin from *Phaseolus vulgaris*) (Corrier and De Loach, 1990). Coloured chicken were injected intra-dermally between 3rd and 4th toe of the right foot or on the wattle with 0.1 mg PHA-P in 0.1 ml of PBS (1 mg PHA-P/ml of PBS). The left foot received 0.1 ml of PBS and served as

control. The thickness of inter-digital skin was measured using micrometer (AMES) at 0 and 24 hr after injection. The skin swelling was calculated by subtracting the skin thickness at 0 hr from that of after 24 hour of injection. The foot web index (FWI) or wattle index was determined as the difference between inter-digital and wattle swelling values of PHA-P injected and control foot or wattle.

Statistical Analysis

Data were subjected to one-way analysis of variance in a completely randomized design (Snedecor and Cochran 1994) using Statistical Package for the Social Sciences (SPSS, 2011). Homogenous subsets were separated using multiple range test described by Duncan (1955). Differences among treatments were considered to be significant when $P \leq 0.05$.

Result and Discussion

Proximate Analysis of Feed and Shatavari Root Meal

The proximate analysis of broiler starter, broiler finisher feed and shatavari root meal has been tabulated in Table 1. The proximate values of Shatavari root meal were in order as reported by Berhane (2000), Kar and Choudhary (1994) and Kumari and Gupta (2016). The proximate values of broiler starter and finisher feed were in the same ranges as reported by Ru *et al.* (2003). The ration was adequate in all the nutrients as per BIS (2007) requirement.

Table 1: Proximate analysis of broiler starter feed, broiler finisher feed and Shatavari root meal

Category	Dry Matter %	Total Ash %	Ether Extract %	Calcium %	Phosphorous %	Protein %	Crude Fibre %
Shatavari	90.33	7.9	0.64	0.24	0.86	4.28	5.62
Broiler Starter feed	88.5	5.35	3.15	1.19	0.69	21.99	3.59
Broiler Finisher feed	88.5	4.94	2.97	1.1	0.59	17.69	3.92

Body Weight

The body weight of coloured chicken from 0-8 weeks of age has been presented in Table 2. There was no significant difference among the different groups in the average weekly body weight during the entire experimental period. However, the T2 group birds had an apparently higher body weight compared to the other treatment groups throughout the experiment. These observations suggested that Shatavari root meal when supplemented in the diet of coloured chicken had no adverse effect on body weight. However, 0.25% level of Shatavari root meal supplemented group birds had an apparently higher body weight compared to the other treatment groups from 2nd week onwards till the end of the experiment. The results collaborate well with the findings of Dahale *et al.* (2014), who also reported that there was no significant difference in treatment groups during 6 weeks trial. However, at 4th week, broilers in 0.25% level of Shatavari root meal

supplemented group grew faster than the birds in groups 0% and 0.5% Shatavari root meal supplemented group. In contrast, other researchers reported increase in body weight with supplementation of *Asparagus racemosus* root powder as a growth promoter at higher level of 0.5, 1 and 1.5% (Rekhate *et al.*, 2004; Pedulwar *et al.*, 2007; Bhardwaj *et al.*, 2009). Mane *et al.* (2012) reported that the weight of chickens increased after feeding with Shatavari root meal at the level of 10 kg/t of feed compared to the control group for 42 days. Pandey *et al.* (2013) reported that the body weight of broiler chicken was increased after feeding with the medicinal plants, such as Ashwagandha (*Withania somnifera*), Shatavari (*Asparagus racemosus*) and Kapikachhu (*Mucuna pruriens*).

Table 2: Effect of dietary supplementation of Shatavari root meal on the average weekly body weight (g) of coloured chicken during 0-8 weeks of age

Treatment	Day Old	1 st wk	2 nd wk	3 rd wk	4 th wk	5 th wk	6 th wk	7 th wk	8 th wk
T1	34.6	92.73	194.47	328.27	555.93	807.77	1054.43	1288.86	1571.8
T2	34.43	91.6	221.27	394.8	640.73	896.4	1143.3	1380.06	1697.5
T3	34.4	93.33	212.13	386.93	626.67	868.73	1101.8	1339.93	1636
T4	34.53	96.07	209.2	375.73	605.13	851.57	1093.83	1343.53	1550.52
T5	34.27	91.67	206.5	347.47	593.83	829.1	1073.13	1309.87	1584.47
T6	34.4	89.2	190.6	321.73	534.13	773	1002.9	1241.93	1509.47
T7	34.57	95.2	209.73	361.47	600.73	855.8	1081.7	1321.23	1588.8
Pooled SEM	0.05	0.99	3.26	8.11	12.2	14.28	14.17	15.9	22.39
Sig Level	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS: Not Significant ($P > 0.05$) SEM: Standard Error of Means.

Feed Conversion Ratio

The weekly feed conversion ratio of coloured chicken from 0-8 weeks of age has been shown in Table 3. Results indicated that T2 coloured chicken had a significantly better ($P < 0.05$) feed conversion ratio than T1, T6, T7 during 2nd week.

Table 3: Effect of dietary supplementation of Shatavari root meal on the average weekly feed conversion ratio (FCR) of coloured chicken during 0-8 weeks of age

Treatment	1 st wk	2 nd wk	3 rd wk	4 th wk	5 th wk	6 th wk	7 th wk	8 th wk
T1	1.54	2.29 ^c	2.69	2.09	2.47	2.53	2.94	2.86
T2	1.58	1.70 ^a	2.08	1.99	2.41	2.57	3	2.69
T3	1.52	1.82 ^{ab}	1.94	2.03	2.6	2.68	2.89	2.75
T4	1.45	1.92 ^{ab}	2.16	2.12	2.46	2.59	2.75	4.22
T5	1.55	1.83 ^{ab}	2.62	1.99	2.72	2.58	2.91	2.96
T6	1.64	2.13 ^{bc}	2.66	2.2	2.55	2.63	2.89	3.02
T7	1.49	2.06 ^{bc}	2.34	2.04	2.43	2.73	2.88	3.08
Pooled SEM	0.03	0.05	0.09	0.04	0.04	0.04	0.05	0.17
Sig level	NS	$P < 0.05$	NS	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly ($P < 0.05$)

NS: Not Significant ($P > 0.05$) SEM: Standard Error of Mean

Further, feed conversion ratio was comparatively higher in the Shatavari root meal supplemented groups than the control group throughout the experiment. The results of the present study are in accordance with the findings of Dahale *et al.* (2014), who reported that at the end of 6th week, 0.25% and 0.5% Shatavari root meal supplemented group showed significantly ($P < 0.05$) better FCR as compared to 0% (control) group.

Blood Biochemical Parameters

Effect of Shatavari root meal supplementation in feed on total plasma protein, total plasma cholesterol, plasma uric acid, plasma GOT (Glutamate Oxaloacetate Transaminase), plasma GPT (Glutamate Pyruvate Transaminase) and plasma ALP (Alkaline Phosphatase) have been presented in Table 4.

Table 4: Effect of dietary supplementation of Shatavari root meal on blood biochemicals (protein, uric acid, SGOT, SGPT, cholesterol and alkaline phosphatase) of coloured chicken at 8 weeks of age

Treatment	Protein (g/dL)	Cholestrol (mg/dl)	Uric acid (mg/dl)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
T1	5.26 ^a	153.815 ^b	4.88	972.48 ^b	5.3	30.35
T2	5.13 ^a	138.24 ^{ab}	4.42	1165.48 ^b	4.57	27.85
T3	5.15 ^a	133.02 ^a	4.63	1118.92 ^b	4.12	28.58
T4	6.17 ^b	131.32 ^a	3.73	1023.32 ^b	4.42	23.87
T5	6.23 ^b	126.04 ^a	4.28	1049.09 ^b	4.27	31.23
T6	6.61 ^b	124.81 ^a	5.41	345.10 ^a	3.98	27.26
T7	6.65 ^b	120.69 ^a	3.86	357.30 ^a	3.39	29.17
Pooled SEM	0.12	132.56	0.16	70.03	0.35	0.73
Sig Level	$P < 0.01$	$P < 0.01$	NS	$P < 0.01$	NS	NS

Means bearing different superscripts within a column differ significantly ($P < 0.01$), ($P < 0.05$)

NS: Not significant ($P > 0.05$) SEM: Standard Error of Means

Statistical analysis of data revealed that plasma protein was significantly higher ($P < 0.05$) in T4, T5, T6 and T7 than T1, T2 and T3. The results of the present study are in accordance with the findings of Kant *et al.* (2014), who reported that biochemical parameters like total serum protein were significantly ($P < 0.05$) higher in 1.5% Shatavari root powder + 200 mg/kg feed vitamin E supplemented group than control group. Data on plasma uric acid indicated that there was no significant difference in plasma uric acid among the treatment groups at 8 weeks of age. Plasma cholesterol levels was significantly higher ($P < 0.05$) in T1 than T3, T4, T5, T6 and T7 at 8 weeks of age in coloured birds. The results of the present study are in accordance with the findings of Kant *et al.* (2014), who reported that biochemical parameters like cholesterol, alanine aminotransferase and aspartate aminotransferase were significantly ($P < 0.05$) lower in 1.5% Shatavari root powder + 200 mg/kg feed vitamin E supplemented group than control group respectively. Bhosale *et al.* (2012) reported that addition of *Asparagus racemosus* root powder at 5 g% and 10 g% levels as feed supplement reduced the plasma cholesterol levels in hyperlipidemic rats. It was also noted that the phytosterol and saponin contents of *Asparagus racemosus* root (besides polyphenols, flavonoids and

ascorbic acid could be responsible for decreased cholesterol levels in the hyperlipidemic rats. Phytosterols compete and displace cholesterol from the intestinal bile acid micelles and in this way decrease the cholesterol circulation in rats. There was no significant difference in total plasma GOT (plasma glutamate Oxaloacetate Transaminase) values among the treatment groups. There was no significant difference in the total plasma GPT (plasma glutamate pyruvate transaminase) values among the treatment groups, at 8 weeks of age in coloured birds at 8 weeks of age in coloured birds. ALP values were significantly lower ($P<0.01$) in T6 and T7 compared to other treatment groups at 8 weeks of age in coloured birds. This shows the hepatoprotective effect of Shatavari root meal when supplemented at higher levels.

Humoral Immune Response

The humoral immune response (response to 1% SRBC HA titre) of the treatment groups have been compiled in Table 5. There was no significant difference was observed in HA and IgM response to 1% SRBC (log₂ titre) among the various treatment groups. Further, the HA and IgM response to 1% SRBC was comparatively better in the T6 and T2 group respectively compared to the other treatment groups. The results of the present study revealed that there was no adverse effect of Shatavari root meal on the immune system of coloured chicken.

Table 5: Effect of dietary supplementation of Shatavari root meal on the humoral immune responses [antibody titer (log₂) values] to 1% SRBC and cell mediated immune response (Foot Web Index) to PHA-P in coloured chicken at 8 weeks of age

Treatment	Total immunoglobulins	IgG	IgM	Foot web index
T1	8.33	2.67	5.67	0.41 ^{ab}
T2	9.67	3.33	6.33	0.69 ^{abc}
T3	8.5	3.5	5	1.01 ^c
T4	7.17	2.5	4.67	0.48 ^{ab}
T5	7.5	4.5	3	0.29 ^a
T6	10	5.5	4.5	0.83 ^{bc}
T7	6.2	2.4	3.8	0.53 ^{ab}
Pooled SEM	0.42	0.39	0.36	0.06
Sig Level	NS	NS	NS	$P<0.01$

Means bearing different superscripts within a column differ significantly ($P<0.01$)

NS: Not Significant ($P>0.05$) SEM: Standard Error of Means

Cell Mediated Immune Response

Effect of Shatavari root meal feeding on cell mediated immune response to PHA-P was determined as FWI (Foot Web Index). T3 group birds had a significantly higher ($P<0.01$) foot web index compared to T1, T4, T5 and T7 (1.01 vs. 0.41, 0.48, 0.29 and 0.53) in response to PHA-P (Table 5). Further, T2, T3, T4, T6 and T7 had comparatively better foot web index compared to control. The results of the present study are in accordance with the findings of Kumari *et al.* (2012), where in it was reported that dietary supplementation of *Asparagus racemosus* extract treated feed had significant ($P<0.01$) increase in cell mediated immune

response of broilers. In addition, similar observations were also reported by Kuttan and Kuttan (1992) in Swiss albino mice.

Conclusion

Thus, it may be concluded that dietary supplementation of Shatavari meal @ 0.5% and above may reduce plasma cholesterol in coloured chicken. Further, dietary supplementation of Shatavari root meal @ 0.5% may improve growth performance and immunity in coloured chicken.

Acknowledgements

The authors are thankful to Vice Chancellor, DUVASU, Mathura for providing the necessary facilities for carrying out this study.

References

1. Berhane M. 2000. Studies on feeding some indigenous galactopoietics feed supplement on performance of crossbred cows, M.Sc. thesis. Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur.
2. Bhardwaj RK, Singh SK, Kumar A and Kumar S. 2009. Performance and haematobiochemical profile of Japanese quails fed *Asparagus recomposes* root powder. Indian Journal of Animal Production and Management. 25: 94-96.
3. Bhosale RR, Kishor JB, Jaju S, Padwal L, Jadhav RR and Deshmukh VS. 2012. Lipid lowering and antioxidant potential of *Asparagus racemosus* in hyperlipidemic rats. International Journal of Basic and Clinical Pharmacology. 1: 168-173.
4. BIS. 2007. Indian Standard: Poultry Feed Specification. V Revision, Manak Bhawan, 9, Bahadurshah Zafar Marg, New Delhi.
5. Corrier DE and Deloach JR. 1990. Evaluation of cell mediated, cutaneous basophil hypersensitivity in young chickens by interdigital skin test. Poultry Science. 69: 403-408.
6. Dahale, GS, Wankhede SM and Kale VR. 2014. Growth performance, serum biochemical profile and carcass quality of broiler chickens fed diets supplemented with Shatavari (*Asparagus racemosus*) root powder. Indian Journal of Animal Nutrition. 31: 166-71.
7. Dahale, GS, Wankhede, SM and Kale, VR. 2014. Growth performance, serum biochemical profile and carcass quality of broiler chickens fed diets supplemented with Shatavari (*Asparagus racemosus*) root powder. Indian Journal of Animal Nutrition. 31: 166-71.
8. Duncan DB. 1955. Multiple range and multiple F tests. Biometrics. 11: 1-42.
9. Kant S, Ali N, Chandra G, Siddique RA and Imran M. 2014. Effect of shatavari and vitamin E on hemato-biochemical profile of broilers during winter season. Veterinary World. 7: 948-951.
10. Kant S, Ali N, Chandra G, Siddique RA. and Imran M. 2014. Effect of shatavari and vitamin E on hemato-biochemical profile of broilers during winter season. Veterinary World. 7: 948-951.
11. Kar A and Choudhary BK. 1994. Important mineral content of few ayurvedic herbs with a discussion on medicinal aspect. Indian drugs. 31: 127-128.
12. Kumari S and Gupta A. 2016. Nutritional composition of dehydrated ashwagandha, shatavari, and ginger root powder. International Journal of Home Science. 2: 68-70.
13. Kumari R, Tiwary BK, Prasad A and Ganguly S. 2012. *Asparagus racemosus* wild. Root Extract as herbal nutritional supplement for poultry. *Global Journal of Research on Medicinal Plants and Indigenous Medicine*. 1: 1-6.
14. Kuttan G and Kuttan R. 1992. Immunomodulatory activity of a peptide isolated from *Viscum album* extract. Immunological Investigations. 21: 285-296.



15. Mane AG, Kulkarni AN, Korake RL and Bhutkar SS. 2012. Effect of supplementation of Ashwagandha (*Withaniasomnifera*) and Shatavari (*Asparagus racemosus*) on growth performance of broilers. *Research Journal of Animal Husbandry and Dairy Science*. 3: 94-96.
16. Martin A, Gross WB and Siegel PB. 1989. IgG and IgM responses in high and low antibody selected lines of chickens. *Journal of Heredity*. 80: 249-252.
17. Pandey NK., Singh DP and Ram N. 2013. Broiler characteristics, sensory qualities. And economics efficiency in Vencobb - 400 chicks supplemented with a conjugated herbal feed additive in diet. *Animal Science Reporter*. 7: 128-132.
18. Pedulwar SN, Zanzad AA, Choudhari AJ, Ramteke BN and Deshmukh GB. 2007. Effect of dietary supplementation of Shatavari (*Asparagus racemosus*) on broilers. *The Indian Journal of Field Veterinarians*. 3: 28-29.
19. Rekhate DH, Ukey S and Dhok AC. 2004. Performance and hemobiochemical profile of broilers feed on supplementation of Shatavari (*Asparagus racemosus*) root powder. *Indian Journal of Poultry Science*. 39: 182-184.
20. Ru YJ, Hughes RJ, Choct M and Kruk JA. 2003. Variation in Nutritive Value of Commercial Broiler Diets. *Asian-Australasian Journal of Animal Science*. 16: 830-836.
21. Sharma S., Dahanukar S and Karandikar SM. 1986. Effects of long term administration of the roots of Ashwagandha (*Withania somnifera*) and Shatavari (*Asparagus racemosus*) in rats. *Indian Drugs*. 23: 133-139.
22. Siegel PB and Gross WB. 1980. Production and persistency of antibodies in chickens to sheep erythrocytes. *Directional selection*. *Poultry Science*. 59: 1-5.
23. Snedecor GW and Cochran WG. 1994. *Statistical Methods*. 9thed. The Iowa, State University Press, Ames, Iowa.
24. SPSS. 2011. *Statistics Version 20.0*. IBM SPSS Inc., USA
25. Van der zijpp AJ. 1983. The effect of genetic origin, source of antigen and dose of antigen on the immune response of cockerels. *Poultry Science*. 62: 205-211.

