Scientific Papers. Series D. Animal Science. Vol. LXV, No. 2, 2022
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

AGE-RELATED CHANGES IN PERFORMANCE, PLASMA PROTEINS AND NITROGEN CONTENT OF EXCRETA IN ROSS 308 BREEDERS

Anca GHEORGHE[1], Mihaela HĂBEANU[1], Nicoleta Aurelia LEFTER[1], Lavinia IDRICEANU[2], Minodora TUDORACHE[2], Ioan CUSTURĂ[2]

[1]National Research-Development Institute for Biology and Animal Nutrition Balotesti, Ilfov, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding authors emails: anca.gheorghe@ibna.ro; minodora.tudorache@usamv.ro

Abstract

The study aimed to evaluate the effect of age on breeders' performance, plasma proteins and total nitrogen content of excreta. The trial was conducted on 150 Ross 308 female breeders' (21-week-old). During 6-weeks, the birds were reared in similar management conditions and fed with standard feeds according to age (21 to 24-weeks, and >25 weeks). At the end of weeks 22, 24 and 26, blood and fresh excreta were sampled for analysis. Plasma proteins were determined by dry chemistry using reagent strips. Total nitrogen from excreta was assessed according to the Kjeldahl method. As expected, age significantly affected body weight, feed intake, protein intake, and nitrogen intake of breeders (P<0.0001). A dynamic age-related change was noticed in breeders' plasma proteins by increasing the total protein, albumin, albumin/globulin ratio, uric acid and urea nitrogen concentrations (P<0.05). The total nitrogen content of excreta registered a tendency to increase at 26-weeks compared to 22-weeks of age (P<0.06). Significant positive correlations were found between certain performance variables (feed intake, protein and nitrogen intake), plasma proteins and excreta total nitrogen.

Key words*: age, excreta nitrogen, performance, plasma proteins, Ross 308 breeders.*

INTRODUCTION

Blood biochemical parameters, as relevant indicators of status health in chickens, are influenced by numerous factors such as species, genotype, age, sex, physiological (breeding, moulting, etc.), nutritional, as well as breeding conditions (Tóthová et al., 2019; Gheorghe et al., 2017; Toghyani et al., 2010; Silva et al., 2007). Blood proteins are an essential indicator of birds' production traits, and their evaluation allows for the identification of metabolic alterations (Filipović et al., 2007).

The assessment of the blood protein concentrations in poultry is also important due to the physiological roles in the body and the maintenance of homeostasis (Piotrowska et al., 2011). Primarily synthesized in the liver, serum proteins control blood volume and pH buffer capacity, transport hormones and medications, helps with cell coagulation, act as enzymes and hormones, and protect the immune system (Melillo, 2013). It was noticed that compared to mammals, the total protein concentrations in birds represent about half of the values (~40 g/l), caused by the higher blood concentrations of osmotically active glucose, which reduce the protein concentrations to maintain the colloid osmotic pressure (Scanes, 2015).

Other important factors that affect the intensity of metabolism and induce changes in poultry blood proteins are age and the associated production (e.g., growing, meat-type) or reproduction processes (Tóthová et al., 2019; Filipović et al., 2007; Szabó et al., 2005).

Pullets' breeders are reared under feed restriction programs to control excessive growth during the rearing phase and avoid compromised health and impaired reproduction (Carneiro et al., 2019; Decuypere et al., 2010).

Birds use approximately 30-50% of the ingested nitrogen during their metabolism, and the excreted part is the potential source of ammonia emission (Such et al., 2021). On the other hand, it is known that poultry farming is responsible for the emission of several air pollutants, including nitrogen (De Sousa et al., 2017; Nahm, 2007). Several factors such as species, age, live weight, dietary composition,

housing conditions or manure management may affect this process (Battye et al., 1994). The current study aimed to evaluate the age-related changes in performance, plasma proteins and total nitrogen content of excreta on Ross 308 breeders.

MATERIALS AND METHODS

Birds and experimental design

The procedures implying live birds and the protocol trial used (no. 366/2021) were allowed by the National Research-Development Institute for Biology and Animal Nutrition (INCDBNA-Balotesti, Romania) Ethical Committee, in line with EU legislation (Directive 2010/63/EU; OJEU, 2010).

The evaluation trial was performed on 150 clinically healthy breeding females (Ross 308) for six weeks (21 to 26 weeks old). The birds were bred in controlled microclimate conditions in an experimental floor hall with wood saving litter, in pens (25 birds/pen), six replicate pens equipped with manual feeders, a nipple drinker line, and nest boxes. The photoperiod and lighting intensity were provided according to the strain management guide (Ross-Aviagen, 2019). The birds were immunized following the specific sanitary-veterinary protocol before the transfer age (20 weeks), and no veterinary treatment was applied during the test period.

The birds were fed with the same standard combined feed according to age: pullets (21 to 24-weeks of age) and breeders (>25 weeks). The feeds were given in the granular form, restricted, daily at 07:30, and water was *ad libitum*. The administered feed was weighed and registered daily. The birds body weight was measured weekly by individually weighing, and weight gain, protein intake (PI) and nitrogen intake (NI) were calculated. Table 1 presents the analyzed nutrient content of the standard feeds used.

Table 1. Analysed composition of breeders' diets

Nutrients (as-fed basis)	Pullets (21-24 weeks)	Breeders (>25 weeks)
Metabolizable energy (MJ/kg) [c]	11.66	11.30
Dry matter	90.35	89.29
Crude protein	14.50	15.30
Total lysine	0.68	0.70
Digestible lysine	0.58	0.60
Total methionine + cysteine	0.64	0.64
Digestible methionine + cysteine	0.56	0.57
Calcium	1.20	2.80
Available phosphorus	0.35	0.40
Crude fibre	6.65	5.30
Neutral detergent fibre	18.33	14.74
Acid detergent fibre	6.86	6.17
Ether extract	4.50	4.10
Ash	7.13	9.91

[c] calculated values

Blood sampling and analysis

Blood was sampled at 22, 24 and 26-weeks of age from 18 birds (3 female/pen of 6 replicates for each age period). Approximately 6 mL of blood per bird was taken via the brachial vein into heparinised tubes in the morning. After blood centrifugation for 15 min. 3000 × g at 4ºC to separate plasma was transferred into 1.5 mL tubes and kept at −20ºC for further analysis. The plasma proteins (total protein, TP; albumin, Alb; uric acid, UA; urea nitrogen, UN) were determined using dry chemistry and reagent strips (Spotchem EZ SP-4430, Arkray Inc., Japan). Each plasma sample was analysed in duplicate for the biochemical variables. Calculated plasma parameters were globulins (Glb) as a difference between TP and Alb and the Alb/Glb ratio.

Excreta sampling and analysis

Excreta samples were collected at 22, 24 and 26-weeks of age from each pen, from 8-10 different places to obtain a pooled sample. After the excreta sub-samples from each pen were homogenised, the representative excreta pooled sample was deposited at −20ºC for N

analysis. The N content was determined according to OJEU (2009) by the Kjeldahl method using a semiautomatic analyser (Kjeltec Auto 1030, Hillerod, Denmark) as described by Hăbeanu et al. (2020).

Statistical analysis

Data were analysed by one-way ANOVA using the GLM procedure (SPSS v.20, 2011). The experimental unit for growth performance parameters was a replicate pen and each sample for the other variables. The responses are presented as means and SEM (standard error of the mean). Pearson's correlation was used to determine the relationship between certain variables. Statistically significant differences were assumed when $P<0.05$.

RESULTS AND DISCUSSIONS

Growth performance

The effect of age on growth performance parameters of Ross 308 female breeders is presented in Table 2. As expected, the results showed significant age-related changes from 21 to 26 weeks in body weight (2240 vs 3206 g/bird), feed intake (126 vs 153 g/bird/day), protein intake (18.27 vs 23.46 g/bird/day) and nitrogen intake (2.92 vs 3.75 g/bird/day) of female breeders ($P<0.0001$).

The breeder's growth performance was comparable with the recommended genetic strain guide (Ross-Aviagen, 2016).

Plasma protein profile

Our results showed a dynamic age-related change in the plasma protein profile of breeders (Table 3).

The relative levels of the total protein, albumin and albumin/globulin ratio showed a gradual and significant increase, with the lowest mean value at 22 weeks (3.22 g/dl TP; 1.59 g/dl Alb; 0.98 Alb/Glb ratio) and the highest at 26 weeks of age (4.23g/dl TP; 2.63 g/dl Alb; 1.65 Alb/Glb ratio; $P<0.05$).

Table 2. Effect of age on growth performance parameters of Ross 308 female breeders

Variables	Age (weeks)						SEM	*P*-value
	21	22	23	24	25	26		
Bodyweight (g/bird)	2240^{f}	2446^{e}	2650^{d}	2846^{c}	3033^{b}	3206^{a}	81.47	*0.0001*
Weight gain (g/bird)	175	206	204	196	188	172	14.86	*0.987*
Feed intake (g/bird/day)	126^{f}	133^{e}	138^{d}	144^{c}	147^{b}	153^{a}	2.22	*0.0001*
Protein intake (g/bird/day)	18.27^{f}	19.24^{e}	20.00^{d}	20.88^{c}	22.49^{b}	23.46^{a}	0.44	*0.0001*
Nitrogen intake (g/bird/day)	2.92^{f}	3.08^{e}	3.20^{d}	3.34^{c}	3.60^{b}	3.75^{a}	0.07	*0.0001*

SEM, standard error of the mean. $^{a\text{-}f}$Means within the row with different superscripts differ significantly (P<0.05).

Table 3. Effect of age on dynamic changes on plasma proteins profile of Ross 308 female breeders

Variables	Age (weeks)			SEM	*P*-value
	22	24	26		
Total protein (g/dl)	3.22^{c}	3.67^{b}	4.23^{a}	0.15	*0.0002*
Albumin (g/dl)	1.59^{c}	2.00^{b}	2.63^{a}	0.15	*0.0001*
Globulin (g/dl)	1.63	1.70	1.60	0.03	*0.290*
Albumin/Globulin ratio	0.98^{c}	1.18^{b}	1.65^{a}	0.10	*0.0001*
Uric acid (g/dl)	0.067^{c}	0.080^{b}	0.092^{a}	0.004	*0.004*
Urea nitrogen (g/dl)	0.021^{b}	0.024^{a}	0.025^{a}	0.001	*0.015*

SEM, standard error of the mean. ^{abc}Means within the row with different superscripts differ significantly (P<0.05).

Uric acid and urea nitrogen concentrations follow the same increasing trend from 0.067 g/dl UA and 0.021 g/dl UN at 22 weeks to 0.092 g/dl UA and 0.025 g/dl UN at 26 weeks of age ($P<0.05$). These variations could be related to the metabolic processes, nutrition changes, and physiological conditions of breeders (Tóthová et al., 2019). Overall, our plasma protein profile results were within normal limits (Ritchie et al., 1994). The blood total protein and albumin are important markers of dietary protein intake and haemoconcentration level (Kraus et al., 2021; Tóthová et al., 2019; Melillo, 2013; Pavlík et al., 2007). A higher total protein value indicates a better health status, and also this might be related to an estrogenic-induced rise in globulin in layers (Marono et al., 2017). Ritchie et al. (1994) stated that proteins are egg yolk precursors (vitellogenin and lipoproteins)

generated in the liver, transferred to the ovary through plasma, and incorporated into egg cells. Evaluating the effects of age, housing system, and genotype on blood parameters and egg quality in Czech and Slovak native hens, Kraus et al. (2021) reported a significant effect of age on total protein concentration. In birds, urea and uric acid are the end-product of protein metabolism, produced by the liver and eliminated by kidneys, considered a biomarker of renal function (Kim et al., 2012; Lumeij, 2008). The uric acid level is influenced by the balance between its production and excretion and is affected by genetic and dietary factors. Our results are in line with other studies (Harlap et al., 2021; Ibrahim et al., 2012) who mentioned that the increased level of uric acid in female birds is related to the ovulatory activities and oviposition, and also genotype had a significant effect (Eleroglu et al., 2015; Isidahomen et al., 2011; Silva et al., 2007).

Excreta nitrogen As shown in Figure 1, the total nitrogen content of excreta registered a tendency to increase at 24- and 26-weeks compared to 22-weeks of age ($P<0.06$), most probably as an effect of dietary protein content. Several studies reported that fed reduced crude protein diets had no adverse impact on the reproductive performance of broiler breeder hens (van Emous et al., 2018; 2015; 2013; Lopez and Leeson, 1995). Previous research reported that uric acid represents 50–60% of poultry excreta's total nitrogen content, which is converted to polluted ammonia (Malomo et al., 2018; Nahm, 2007; Nahm, 2003).

Lopez and Leeson (1995) stated that higher nitrogen excretion is due to ammonia, urea and creatinine elimination, excessive water intake/output, and litter and environmental quality issues in a breeder house. The same authors concluded that nitrogen excretion is directly related to nitrogen intake, so there is the potential to reduce the nitrogen content of manure.

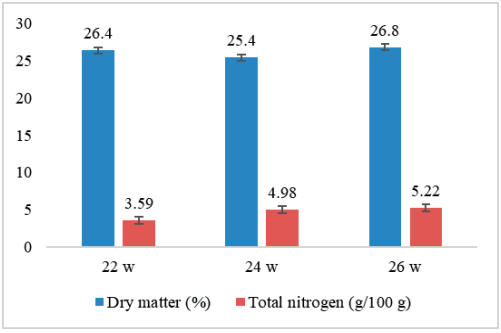

Figure 1. Effect of age on excreta nitrogen of Ross 308 female breeders

Positive significant correlations ($P<0.05$) were found between certain performance variables (feed intake, protein intake, nitrogen intake), plasma proteins and excreta total nitrogen (Table 4).

Table 4. Pearson's correlation between nitrogen excreta, performance and plasma proteins variables

	N g%	FI g/day	PI g/day	NI g/day	TP g/dl	Alb g/dl	Glb g/dl	Alb/Glb	UA g/dl	UN g/dl
N excreta g%	1	0.893** 0.001	0.828** 0.006	0.828** 0.006	0.840** 0.005	0.825** 0.006	0.047 0.905	0.780* 0.013	0.809** 0.008	0.819** 0.007
FI g/day		1	0.983** 0.0001	0.983** 0.0001	0.949** 0.0001	0.966** 0.0001	-0.149 0.702	0.951** 0.0001	0.920** 0.0001	0.890** 0.001
PI g/day			1	1.000** 0.0001	0.952** 0.0001	0.986** 0.0001	-0.245 0.526	0.989** 0.0001	0.907** 0.001	0.837** 0.005
NI g/day				1	0.952** 0.0001	0.986** 0.0001	-0.245 0.526	0.989** 0.0001	0.907** 0.001	0.837** 0.005
TP g/dl					1	0.985** 0.0001	0.038 0.922	0.933** 0.0001	0.827** 0.006	0.748* 0.021
Alb g/dl						1	-0.134 0.730	0.981** 0.0001	0.880** 0.002	0.774* 0.014
Glb g/dl							1	-0.324 0.395	-0.350 0.356	-0.185 0.634
Alb/Glb								1	0.908** 0.001	0.768* 0.016
UA g/dl									1	0.811** 0.008

**Significant correlation at the 0.01 level; *Significant correlation at the 0.05 level.

CONCLUSIONS

The results showed that age-specific changes in Ross 308 breeders' growth performance were positively correlated with protein intake and plasma protein profile, reflecting their good health state and physiological status. Total nitrogen content of excreta registered a tendency to increase with age and reflected the nitrogen intake. Further studies are necessary to develop solutions to reduce nitrogen losses in breeders.

ACKNOWLEDGEMENTS

This study was funded by the Ministry of Agriculture and Rural Development [projects ADER 8.1.9/2019 and ADER 9.1.4/2019], and supported by the Ministry of Research, Innovation and Digitalization [project PFE 8/2021], Romania.

REFERENCES

Battye, R., Battye, W., Overcash, C., & Fudge, S. (1994). *Development and selection of ammonia emission factors*; Final Report; U.S. Environmental Protection Agency: Washington, DC, USA.

Carneiro, P.R.O., Lunedo, R., Fernandez-Alarcon, M.F., Baldissera, G., Freitas, G.G., & Macari, M. (2019). Effect of different feed restriction programs on the performance and reproductive traits of broiler breeders. *Poultry Science*, 98, 4705–4715.

Decuypere, E., Bruggeman, V., Everaert, N., Li, Y., Boonen, R., & de Tavernier, J. (2010). The broiler breeder paradox: ethical, genetic and physiological perspectives, and suggestions for solutions. *British Poultry Science*, 51, 569–579.

De Sousa, F.C., de Tinôco, I.F.F., da Silva, J.N., de Jesus Fôlgoa Baptista, F., Souza, C.F., & Lopes Silva, A. (2017). Gas emission in the poultry production. *Journal of Animal Behaviour and Biometeorology*, 5, 49–55.

Eleroglu, H., Yıldırım, A., Duman, M., & Sekeroglu, A. (2015). The welfare of slow-growing broiler genotypes reared in organic system. *Emirates Journal of Food and Agriculture*, 27, 454–459.

Filipović, N., Stojević, Z., Milinković-Tur, S., Ljubić, B.B., & Zdelar-Tuk, M. (2007). Changes in concentration and fractions of blood serum proteins of chickens during fattening. *Veterinarski Arhiv*, 77(4), 319–326.

Gheorghe, A., Hăbeanu, M., Tabuc, C., Dumitru, M., & Lefter, N.A. (2017). Blood parameters, digestive organ size and intestinal microflora of broiler chicks fed sorghum as partial substitute of corn. *Bulletin UASVM Animal Science and Biotechnologies*, 74(2), 162–168.

Harlap, Y.S., Kadirov, N.N., Gorelik, L.S., Meshcheryakova, G.V., & Mukhamedyarova, L.G. (2021). Age-related variability of indicators of protein metabolism in the blood of laying hens. *IOP Conf. Series: Earth and Environmental Science,* 677, 042019.

Hăbeanu, M., Gheorghe, A., Lefter, N.A., Untea, A., Idriceanu L., & Ranta, M.F. (2020). Assessment of certain nitrogen metabolism indicators, enteric CH4 and CO2 emitted through manure related to different diets in barrow. *Archiva Zootechnica*, 23(2), 129–142.

Ibrahim, A., Aliyu, J., Abdu, M., & Hassan, A. (2012). Effects of age and sex on serum biochemistry values of turkeys (*Meleagris gallopavo*) reared in the semi-arid environment of Nigeria. *World Applied Sciences Journal*, 16(3), 433–436.

Isidahomen, E.C., Ozoje, M.O., & Njidda, A.A. (2011). Haematological and serum biochemical indices of local and exotic chickens in a sub-humid tropical environment. *European Journal of Biological Sciences*, 3, 16–21.

Kraus, A., Zita, L., Krunt, O., Härtlová, H., & Chmelíková, E. (2021). Determination of selected biochemical parameters in blood serum and egg quality of Czech and Slovak native hens depending on the housing system and hen age. *Poultry Science*, 100(2), 1142–1153.

Lopez, G., & Leeson, S. (1995). Nitrogen Content of Manure from Older Broiler Breeders Fed Varying Quantities of Crude Protein. *The Journal of Applied Poultry Research*, 4(4), 390-394.

Lumeij, J.T. (2008). Avian Clinical Biochemistry. In: Kaneko, J.J., Harvey, J.W. and Bruss, M.L., Eds., Clinical Biochemistry of Domestic Animals, 6th Edition, Academic Press, San Diego, 839-872.

Malomo, G.A., Bolu, S.A., Madugu, A.S., & Usman, Z.S. (2018). *Nitrogen Emissions and Mitigation Strategies in Chicken Production*. In Animal Husbandry and Nutrition; Yücel, B., Taskin, T., Eds.; London, UK: Intech Open.

Melillo, A. (2013). Applications of serum protein electrophoresis in exotic pet medicine. *Veterinary Clinics of North America Exotic Animal Practice*, 16(1), 211–225.

Nahm, K.H. (2007). Feed formulations to reduce N excretion and ammonia emission from poultry manure. *Bioresource Technology*, 98(12), 2282-300.

Nahm, K.H. (2003). Evaluation of the nitrogen content in poultry manure. *World's Poultry Science Journal*, 59, 77–88.

OJEU-Official Journal of the European Union L54 (2009). Commission Regulation (EC) No. 152/2009 laying down the methods of sampling and analysis for the official control of feed. Series L 155:1–66.

OJEU-Official Journal of the European Union (2010). Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes. Series L 276:33-79.

Pavlík, A., Pokludová, M., Zapletal, D., & Jelínek, P. (2007). Effects of housing systems on biochemical indicators of blood plasma in laying hens. *Acta Veterinaria Brno*, 76, 339–347.

Piotrowska, A., Burlikowska, K., & Szymeczko, R. (2011). Changes in blood chemistry in broiler chickens during the fattening period. *Folia Biologica* (Kraków), 59, 183–187.

Ritchie, B.W., Harrison, J.G., & Harrison, R.L. (1994). *Avian Medicine*. Florida, USA: Winger's Publishing House.

Scanes, C.G. (2015). *Protein metabolism*. In: Scanes, C.G., editor. Sturkie's Avian Physiology. 6th ed. Academic Press, Elsevier Inc., Waltham, MA. 455-468.

Silva, P.R.L., Freitas-Neto, O.C., Laurentiz, A.C., Junqueira, O.M., & Fagliari, J.J. (2007). Blood serum components and serum protein test of Hybro-PG broilers of different ages. *Brazilian Journal of Poultry Science,* 9, 229–232.

SPSS (2011). Statistics version 20.0. IBM SPSS Inc, USA.

Such, N., Pál, L., Strifler, P., Horváth, B., Koltay, I.A., Rawash, M.A., Farkas, V., Mez″olaki, Á., Wágner, L., & Dublecz, K. (2021). Effect of feeding low protein diets on the production traits and the nitrogen composition of excreta of broiler chickens. *Agriculture*, 11, 781.

Szabó, A., Mézes, M., Horn, P., Sütő, Z., Bázár, G.Y., & Romvári, R. (2005). Developmental dynamics of some blood biochemical parameters in the growing turkey (*Meleagris gallopavo*). *Acta Veterinaria Hungarica*, 53(4), 397-409.

Tóthová, C., Sesztáková, E., Bielik, B., & Nagy, O. (2019). Changes of total protein and protein fractions in broiler chickens during the fattening period. *Veterinary World*, 12(4), 598-604.

van Emous, R.A., Kwakkel, R.P., van Krimpen, M.M., & Hendriks, W.H. (2013). Effects of growth patterns and dietary crude protein levels during rearing on body composition and performance in broiler breeder females during the rearing and laying period. *Poultry Science*, 92, 2091–2100.

van Emous, R.A., Kwakkel, R.P., van Krimpen, M.M., & Hendriks, W.H. (2015). Effects of dietary protein levels during rearing and different dietary energy levels during lay on body composition and reproduction in broiler breeder females. *Poultry Science*, 94, 1030– 1042.

van Emous, R.A., de la Cruz, C.E., & Naranjo, V.D. (2018). Effects of dietary protein level and age at photostimulation on reproduction traits of broiler breeders and progeny performance. *Poultry Science*, 97, 1968–1979.

**Ross-Pocket Guide 2019-2 Production-EN.pdf

**Ross308-PS-PO-EN-2016.pdf