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Report on the activities run during year 2011 within the framework of the project:

"*INFLUENCE OF CONVENTIONAL AND FREE-RANGE FARMING SYSTEMS ON THE NUTRITIONAL-DIETETIC AND SANOGENIC QUALITY OF POULTRY PRODUCTS (MEAT, EGGS) ISSUED FROM GALLUS DOMESTICUS SPECIES*"

Project type: **PN-II-Human resources-PD 2010-2012**
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STAGE 2011
-unique-

Project manager: Assist.prof. Răzvan Mihail RADU-RUSU, PhD

Objective 1 - Investigation of quality of chicken eggs from market / purchased directly from the producer, depending on system growth of laying hens (following form single stage 2010)

Activity 1.1. *Performing chemical analysis under Weende schematics (moisture, dry matter, ash, proteins, lipids). Calculation (determination) of eggs caloricity.*

Acquired biological material was represented by eggs produced by hens in three versions operating system technology and was divided into three groups needed to carry out research, as follows:
- Lot B - 90 eggs from the maintenance system of hens in battery cages, in the halls blind -super-operating system (indicated by number 3 in the code printed on mineral shell eggs);
- Lot S - 90 eggs from hens system maintenance on land, permanent litter the halls blind -intensive operating system (indicated by number 2 in the code printed on mineral shell eggs);
- Lot FR - 90 eggs from hens maintenance system ground, litter standing in the halls that allow birds access to an outdoor paddock housing (indicated by the 1 code number printed on a mineral shell eggs).

After a preliminary cleaning of the mineral shell eggs from the 3 lots of experience, separated whites and yolks of 90 eggs for each batch corresponding to B, S and FR.

Next, we proceeded to the dehydration of biological material in an oven at a temperature of 60 ° C in appropriately labeled containers. Following dehydration, samples were ground to powder as specific analytical techniques used later to determine the gross chemical composition (tab. 1) and caloricity (tab. 2), as well as in different nutrients, such as fatty acids (tab. 3) and cholesterol (tab 4). There were performed by 20 repetitions for each chemical constituent, except for fatty acids and cholesterol (by 5 repetitions).

Table 1

Eggs chemical composition, as related to laying hens husbandry system

Egg compound	Chemical constituents (%)	Group B (n=20)			Group S (n=20)			Group FR (n=20)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Yolk	Water	52.16[a]	±1.37	11.75	52.43[b]	±1.42	12.08	52.87[b]	±1.32	11.17
	D.M.*	47.84	±1.26	11.75	47.57	±1.28	12.08	47.13	±1.18	11.18
	Ash	1.72	±0.03	7.80	1.73	±0.03	7.6	1.69	±0.03	7.94
	T.N.M.*	15.91	±0.32	8.99	15.89	±0.31	8.71	16.08	±0.31	8.62
	Lipids	27.08[b]	±0.45	7.43	26.87[b]	±0.43	7.15	26.14[a]	±0.39	6.67
	N.F.E.*	3.13	±0.05	7.14	3.08	±0.05	6.81	3.22	±0.04	5.56
Albumen	Water	86.93	±2.12	10.91	87.02	±2.15	11.04	87.14	±2.24	11.50
	D.M.	13.07	±0.32	10.92	12.98	±0.32	11.04	12.86	±0.33	11.51
	Ashes	0.71	±0.01	6.30	0.69	±0.01	6.8	0.63	±0.01	7.10
	T.N.M.	11.2	±0.23	9.18	11.21	±0.23	9.21	11.4	±0.21	8.24
	Lipids	0.18	±0.004	9.94	0.15	±0.003	10.01	0.13	±0.003	10.32
	N.F.E.	0.98	±0.02	9.13	0.93	±0.02	11.26	0.70	±0.02	12.78
Whole egg	Water	74.98[a]	±1.08	6.44	75.09[b]	±1.07	6.4	75.62[b]	±1.08	6.39
	D.M.	25.02	±0.36	6.43	24.91	±0.36	6.4	24.38	±0.35	6.38
	Ashes	1.14	±0.02	7.85	1.11	±0.02	7.91	1.09	±0.02	8.21
	T.N.M.	12.38	±0.21	7.59	12.44	±0.21	7.53	12.56	±0.18	6.41
	Lipids	10.45[b]	±0.17	7.28	10.28[b]	±0.17	7.49	9.81[a]	±0.19	8.66
	N.F.E.	1.05	±0.02	8.52	1.08	±0.02	8.67	0.92	±0.02	9.72

ANOVA: [ab]different superscripts reveal significant statistical differences ($p<0.05$) between groups
* D.M. = dry matter; T.N.M.=total nitrogen matters; N.F.E.=nitrogen free extract

Regarding the chemical composition of eggs (Table 1), namely the yolk, and statistically significant differences were observed on the water and dry matter content of eggs from free-range system other two conventional. Protein content varied between 15.89 to 16.08% and 26.14 lipid between limits (FR) - 27.08% (B), given also statistically significant for the latter parameter. If the white, analytical values were close between the three systems analyzed no cases of significant difference (SU = 12.87 to 13.07% from 11.20 to 11.40% = TNM, Fat = 0 0.13 to 0, 18%). Influenced the chemical composition of yolk but the results determined for the whole egg, respectively from 24.38 to 25.02% DM (FR vs. dif. significant. S and B),12.38 to 12.56% TNM, 9.81 to 10.45% fat (FR vs. dif. significant. S and B). This dynamic led to the chemical constituents caloricity values (Table 2) of 174.25 ±3.26 g egg mass Kcal/100 in group B, 173.10 ± 3.16 g egg mass Kcal/100 the group S , 168.65 ± 2.97 respectively Kcal/100 g egg mass in FR group were seen in the therefore slightly improved properties, in terms of diet, of eggs from free range system.

Table 2

Eggs caloric value, as related to laying hens husbandry system

Caloricity per egg compartment	Group B (n=20)			Group S (n=20)			Group FR (n=20)		
	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Kcal /100 g yolk	361.09	±7.92	9.81	358.77	±7.81	9.74	353.51	±7.63	9.65
Kcal /100 g albumen	69.67	±1.09	7.00	69.23	±1.06	6.82	69.16	±1.02	6.60
Kcal /100 g egg mass	174.25	±3.26	8.37	173.10	±3.16	8.17	168.65	±2.97	7.88
Kcal//whole egg of 60 g	105.00	±1.97	8.37	103.86	±1.87	8.05	101.00	±1.78	7.88

Activity 1.2. *Test for eggs content in fatty acids and cholesterol (gas/ liquid chromatography)*

There were used the analytical standards AOAC 971.11-gas chromatography for fatty acids and AOAC 941.09-titrimetry for cholesterol content. Improved nutritional properties arising from the fatty acid profile of the samples analyzed (Table 3), the eggs produced in free-range system, the balance of saturated FA: monounsaturated FA: polyunsaturated FA tilted slightly in favor of PUFA (SFA: MUFA: PUFA = 1.66:1.94:1 in group B to group S 1.62:1.92:1;1.59:1.92:1 the FR group).

Table 3

Fatty acids content of the eggs produced by hens in different husbandry systems

Fatty acids profile	M.U.	Group B		Group S		Group FR	
		Yolk	Whole egg	Yolk	Whole egg	Yolk	Whole egg
Saturated FA:	g/100g	**9.531**	**3.118**	**9.512**	**3.096**	**9.483**	**3.054**
12:0	g/100g	0.009	0.002	0.009	0.001	0.007	0.001
14:0	g/100g	0.101	0.031	0.099	0.03	0.095	0.027
16:0	g/100g	6.818	2.218	6.728	2.201	6.681	2.182
18:0	g/100g	2.386	0.794	2.361	0.788	2.254	0.767
Monounsaturated FA:	g/100g	**11.681**	**3.647**	**11.694**	**3.665**	**11.725**	**3.682**
16:1	g/100g	0.862	0.199	0.871	0.201	0.883	0.205
18:1	g/100g	10.304	3.378	10.323	3.38	10.351	3.381
20:1	g/100g	0.009	0.001	0.01	0.001	0.012	0.002
22:1	g/100g	0.010	0.001	0.011	0.002	0.015	0.004
Polyunsaturated FA:	g/100g	**4.198**	**1.882**	**4.209**	**1.907**	**4.226**	**1.917**
18:2	g/100g	0.347	1.410	0.352	1.418	0.367	1.429
18:3	g/100g	0.101	0.042	0.116	0.044	0.124	0.047
20:4	g/100g	0.409	0.175	0.418	0.179	0.427	0.181
20:5 ω-3	g/100g	0.008	0.002	0.011	0.003	0.019	0.005
22:5 ω-3	g/100g	0.003	0.006	0.004	0.008	0.007	0.011
22:6 ω-3	g/100g	0.103	0.051	0.109	0.054	0.118	0.062
SFA:MUFA:PUFA		**2.27:2.78:1**	**1.66:1.94:1**	**2.26:2.78:1**	**1.62:1.92:1**	**2.24:2.77:1**	**1.59:1.92:1**

The same trend that better dietary properties for eggs produced in free-range farm type was maintained and cholesterol content in egg mass resulting 373.17 µg/100 g for eggs FR, compared to 397.04 µg/100 g group C or 386.36 µg/100 g in group S(Table 4).

Table 4

Eggs cholesterol content, as related to laying hens husbandry system

Cholesterol in egg compounds	Group B (n=20)			Group S (n=20)			Group FR (n=20)		
	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
µg/100 g yolk	1094	±24.71	10.10	1073	24.30	10.13	1065	±23.98	10.07
µg/100 g albumen	ND	-	-	ND	-	-	ND	-	-
µg/100 g egg mass	397	±6.83	7.69	386	6.65	7.71	373	±6.25	7.49
µg//whole egg of 60 g	238.20	±4.10	7.69	231.6	3.99	7.71	223.80	±3.75	7.49

ND: undetectable

Although these data show, at least in terms of nutritional advantage eggs from free-range farms on the growth of conventional systems, the results are not conclusive, knowing that the material came from different farms technology, in which probably have used different food recipes which influenced, rather than technological system itself, the profile of chemical constituents of the egg.

Moreover, these data must be supplemented in future research, the results of consumer safety, the free-range system known predisposition to produce eggs with greater amounts of pollutants or pathogens transferred from the environment, where birds have access the day.

Objective 2 - Make a documentation / training in a research institution in the European Union, within the quality of animal products field

Activity 2.1. *Documentation on the influence of applied poultry farming systems on meat quality*

Activity 2.2. *Documentation on the influence of applied poultry husbandry system on quality of eggs*

Activity 2.3. *Training in laboratory techniques for evaluating the quality of poultry products by invasive and non-invasive methods*

During the mobility I had the opportunity to use the tools of documentation provided by the INRA center (library, digital databases, experimental protocols poultry team). I also had the opportunity to learn and apply techniques from November borate(electrophoresis, zimography, PCR testing quality mineral shell eggs and egg proteomics tests texture and technological properties of meat from poultry.

I also had the opportunity to participate in several meetings of research teams working on the quality of meat and eggs, to know the latest trends in French poultry research and elaborate some further scientific collaboration, and some ideas on future topics research in poultry specialists team of Veterinary Medicine Iasi.

To describe these activities as evidence of achieving this goal during June-July 2011, attached to the facsimile letter of certification issued by Mr. Dr. Yves Nys HC, research director of the Poultry Research Unit Nouzilly INRA, Tours, France.

I want to express my gratitude in this way to express agreement on making this mobility.

INRA

Institut National de la Recherche Agronomique

Objet : Lettre d'**attestation** pour le séjour du Dr Radu Rusu à l'unité de Recherches Avicoles
INRA, Fr 37380 Nouzilly

Nouzilly, le 7 Juillet 2011

Cher Monsieur, Madame

Nous avons été très heureux d'accueillir le Dr. Razvan Mihail Radu Rusu, Assistant professeur à la Faculté de Zootechnie, à l'Université de Sciences Agricoles et Médecine Vétérinaire de Iasi (USAMV Iasi) pour la période du 7 Juin au 7 Juillet 2011, dans l'Unité de Recherches Avicoles (UR83) de l'Institut National de Recherche Agronomique – INRA de Tours-Nouzilly. Ce séjour postdoctoral de 31 jours a permis au Dr Radu Rusu de participer à différentes expériences et d'acquérir de nouvelles méthodes expérimentales au sein des laboratoires de l'équipe travaillant sur la Qualité de l'œuf – Fonction et régulation des protéines de l'œuf (FRPO) et de l'équipe Qualité des viandes de volaille. Les principales activités au cours de ce séjour ont concernées:

- La recherche de documentation en utilisant les outils mise à disposition de l'unité par l'INRA: consultation des bases de données à partir de ISI Web of Knowledge sur des sujets variés – qualité des œufs, des ovoproduits, de la viande et systèmes conventionnels et alternatifs d'élevage des espèces avicoles.
- Echange quotidien avec des chercheurs de l'unité sur les différents programmes de recherches

Et sur un plan technique de laboratoire :

- électrophorèse en gel de polyacrylamide pour l'identification de protéines de l'œuf.
- zymographie en gel de polyacrylamide pour identifier des enzymes actives dans le blanc d'œuf (protéases, antiprotéases).
- Initiation aux techniques de mesures biomoléculaires : PCR en temps réelle, (polymerase chain reaction) réalisé sur différents tissues de poules pondeuses impliqués dans la formation des compartiments de l'œuf (foie pour la formation du jaune, segments de l'oviducte pour la formation du blanc et des membranes coquillières).
- testage de propriétés mécanique de la coquille (résistance à la rupture et déformation).
- Qualité des viandes : découpe anatomique des carcasses de poulets de chair d'élevage biologique pour analyser les rendements de filets et cuisses. Cette méthode décrite sur un Cdrom pourra être utilisé pour l'enseignement.
- manipulations sur les propriétés technologiques influençant la qualité de la viande: pH, texture, force de cisaillement, couleur.

Institut National de la Recherche Agronomique
Etablissement public à caractère scientifique et technologique placé sous la tutelle conjointe des ministres chargés de la recherche et de l'agriculture

Centre de Tours –Unité de Recherches Avicoles, UR83
INRA - 37380 Nouzilly - France - Télécopie : 02.47.42.77-78 - Tél. : 02.47.42.72.82

Ce séjour a été une excellent opportunité pour le Dr. Razvan Mihail Radu Rusu d'approfondir des techniques de laboratoire éprouvées ou innovantes qui lui permettront de poursuivre ses expérimentations et d'introduire de nouveaux protocoles dans les laboratoires de votre université.

Nous avons été très heureux d'accueillir le Dr Radu Rusu au sein de notre unité de Recherches avicoles, de l'initier à de nouvelles techniques de biochimie et de renforcer ainsi la collaboration entre l'équipe de l'USAMV Iasi et notre équipe INRA.

Cordialement,

Dr. H.C. Yves Nys,
Directeur de recherches INRA

Objective 3 - Dissemination of achieved results

Activity 3.1. *Attending of national and international conferences*

The research results have been communicated and discussed with other researchers from the field, in 3 different occasions:

The symposium „Modern animal science – food safety and socioeconomic development" – April 14-15, 2011, UASVM Iaşi.

The XIV Symposium on eggs and eggs products quality and the XX Symposium on poultry meat quality, September 4-8, Leipzig, Germany, organized by the World Poultry Science Association, German branch.

The documentation/training mobility at INRA Tours Nouzilly–Poultry Science Unit, France, during which I activated between 6 June - 8 July 2011 in the research team of Dr. H.C. Yves Nys, President of the European Federation of World Poultry Science Association.

Activity 3.2. *Publication of articles in scientific journals of international circulation, indexed in recognized databases, with high impact factor*

There were developed and published three papers:

Radu-Rusu R.M., Usturoi M.G., Vacaru-Opriş I., 2011 - Influence of conventional and alternative husbandry systems on the poultry meat dietetic value. *Lucrări ştiinţifice USAMV Iaşi, seria Zootehnie, 56 (16):202-206*

Radu-Rusu R.M., Usturoi M.G., Radu-Rusu C.G., Albu A., Vacaru-Opriş I., 2011 - Chemical and dietetic traits of the hen eggs within the free-range and enriched cages farming systems. World Poultry Science Journal, Vol. 67, Supplement, p.97 şi in Proceedings of XIV European Symposium on the Quality of Eggs and Eggs Products and the XX European Symposium on the Quality of Poultry Meat, Leipzig, Germany, http://www.eggmeat-2011.de/

*Usturoi M.G., Radu-Rusu R.M., Lazăr R., 2011 - S*tudies on the welfare condition provided to laying hens within alternative husbandry systems. *Lucrări ştiinţifice USAMV Iaşi, seria Zootehnie, 56 (16):123-126*

In review in ISI journal - Radu-Rusu R.M., Albu A., Radu-Rusu C.G., Usturoi M.G., 2011?-2012? - Effect of Conventional and Alternative Farming Systems on Table Hen Eggs Nutritional Composition, Caloricity and Cholesterol Level, Intl. Journal of Food Science and Technology.

Activity 3.3. *Publishing a website that would include data on nutritional-biological value of poultry products, depending on the farming system. No data related to brands or company names will be included.*
The website has been published and updated, being available at: http://www.uaiasi.ro/PN_2/prodavis/ (RO) or http://www.uaiasi.ro/PN_2/prodavis/en/indexen.html (EN).

Objective 4 Pilot Experiment: laying hens farming in superintensive system (batteries of cages, conventional/improved) and free-range type-qualitative assessment of eggs production during laying onset, peak and plateau moments
Activity 4.1. *The acquisition of biological material (pullets) and accommodation of the tested birds in accordance with technological systems.*

There were used as biological material Lohmann Brown Classic hens hybrid, available in house Biobaza of Veterinary Medicine Iasi, later supplemented with a Flock of 250birds purchased from Avicola Bucharest, Romania's representative on avian genetic material manufacturer Tierzucht Lohmann GmbH, Germany.

Experimental protocol used is shown in the following schedule:

Experimental parameters	Group B	Group CA	Group FR
Biological material	Lohman Brown Classic hens, aged 20-70 weeks		
Flock at onset (hens)	250	250	250
Husbandry system	Superintensive Conventional Standard BP-3 batteries	Superintensive Alternative Improved cages	Semiintensive Alternative Deep litter + acces in ext. paddock
EU agreement from January 1, 2012	NO	YES	YES
Surface/hen	500 cm^2	800 cm^2	1111 cm^2
Feeding	21-5 mixed feed	21-5 mixed feed	21-5 mixed feed
Lighting schedule	According to product guide	According to product guide	According to product guide+natural environment

The experiment was organized by dividing the total flock of birds into 3 groups (groupB - 250 birds conventional cages kept isolated house, lot CA-250 birds reared in cages improved dimensional aspect and equipment (perch), isolated in the hall; FR-250 group raised birds on land, permanent litter the house with access to a grass paddock, for the hall of growth. investigated parameters were followed over 5 times the curve of laying birds correlated with age, expressed in weeks (started 21 weeks,top-27 weeks per plate. 1-40 weeks = goal 4, per set. 2-56 weeks and finally lay - 70 weeks = objective 5). All birds received the same food (training that combined feed for laying at the beginning of the experiment, then mixed fodder 21-5, optimized lay-phase) from a single manufacturer to eliminate differences of food recipe data that can influence, in particular, lipid profile of products, but also the production and shell quality.

Activity 4.2. *Investigations on the physiological status of the studied fowl (haematological profile, blood chemistry).*

Blood was collected by venous puncture from the brachial vein from approx. 12 birds from each group randomly chosen (about 5% of the Flock) and was stored in vacuum containers, hard anticoagulant. Subsequently, measurements were performed to identify blood tests (ABX Micros analyzer using VET ABC) (Table 5) and biochemical parameters (Table 6), related to the theme of the research project, using Accent Cormay analyzer 200 (cholesterol) and liquid chromatography (plasma corticosteronee), this hormone consecutive extraction with organic solvent(petroleumdiethyl ether) according to the method described by Navara and Pinson,2010. were performed by five repetitions for each parameter investigated / lot.

Table 5

Hematological parameters, as related to laying hens husbandry, during laying onset, peak and plateau-stage I

Control moment	Group	RBC (n=5) ($10^6/mm^3$)			PCV (n=5) (%)			MCV (n=5) (μm^3)			MCH (n=5) (pg)			MCHC (n=5) (g/100ml)			WBC (n=5) ($10^3/mm^3$)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Laying onset (21 weeks)	**B**	2.24	0.05	5.29	28.74	0.36	2.77	128.30	2.28	3.97	39.69	0.76	4.28	30.93	0.57	4.13	20.71	0.50	5.43
	CA	2.32	0.07	7.08	29.81	0.59	4.44	128.49	3.26	5.67	35.84	0.64	4.01	27.89	0.60	4.85	21.88	0.70	7.19
	FR	2.31	0.08	8.18	27.73	0.50	4.03	120.04	3.23	6.02	42.48	1.00	5.24	35.39	0.89	5.64	21.94	0.82	8.31
Laying peak (27 weeks)	**B**	2.45	0.07	6.61	28.92	0.45	3.46	118.04	2.62	4.96	43.51	0.94	4.85	36.86	0.81	4.91	23.28	0.70	6.72
	CA	2.78	0.10	7.83	30.08	0.66	4.91	108.20	3.04	6.28	38.19	0.77	4.49	35.30	0.85	5.39	26.18	0.94	8.04
	FR	2.34	0.09	8.47	28.38	0.67	5.31	121.28	3.68	6.79	45.08	1.08	5.34	37.17	1.01	6.07	27.83	1.08	8.69
Plateau stage I 40 weeks)	**B**	2.62	0.08	6.82	29.06	0.44	3.36	110.92	2.49	5.02	45.18	1.02	5.06	40.73	0.92	5.04	22.65	0.70	6.93
	CA	2.86	0.10	8.06	31.25	0.56	3.97	109.27	2.90	5.93	41.26	0.86	4.68	37.76	0.90	5.31	25.62	0.95	8.27
	FR	2.32	0.09	8.74	29.72	0.61	4.58	128.10	3.76	6.56	48.34	1.17	5.43	37.74	1.01	6.00	26.19	1.05	8.97

The number of red blood cells showed typically an upward trend, as the birds have advanced in years, being higher for batch CA to B and FR groups, at all times of control, standing, However, in any event, the normal metabolism of the species studied. A similar dynamic occurred and the number of white blood cells, which are better represented in the sample to the period of plateau and, in any case at a lot higher for FR, possibly due to an immune response to these exalted birds, due to daily exposure to changes in microclimate and etiologic agents in the outside hall(grass paddock), where birds had free access throughout each day. For other haematologic indexes, evolution followed the same trend, with lower values in the early laying and large to plateau.

Stress indicators, such as. serum corticosteronee levels (Table 6) were slightly higher for conventional batteries bird growth (B) compared with other experimental groups(CA and FR), but without being detected significant differences between groups(Table 6) . Thus, serum corticosteronee levels varied between 2.8 ± 0.082 ng / ml plasma (B) - 2.6 ± 0.075 ng / ml plasma (CA) - 2.3 ± 0.068 ng / ml plasma (FR) at baseline lay, then increased to peak at 3.1 ± 0.099 lay ng / ml plasma (B) - 2.9 ±0.091 ng / ml plasma (CA) - 2.7 ± 0.085 ng / ml plasma (FR) to decrease slightly and stabilize during the plateau: 2.9 ± 0.078 ng / ml plasma (B) - 2.7 ± 0.075 ng / ml plasma (CA) - 2.5 ± 0.070 ng / ml plasma (FR).

Table 6

Blood serum biochemical parameters, as related to the hens husbandry system, during laying onset, peak and plateau-stage I

Control moment	Parameters	Group B (n=5)			Group CA (n=5)			Group FR (n=5)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Laying onset (21 weeks)	Serum cholesterol (mg/dl)	23.15	0.79	7.61	22.81	0.76	7.42	22.73	0.76	7.49
	Serum corticosterone (ng/ml)	2.8	0.082	6.58	2.6	0.075	6.47	2.3	0.068	6.63
Laying peak (27 weeks)	Serum cholesterol (mg/dl)	23.74	0.74	6.98	22.93	0.70	6.82	22.86	0.72	7.01
	Serum corticosterone (ng/ml)	3.1	0.099	7.11	2.9	0.091	6.98	2.7	0.085	7.08
Plateau stage I (40 weeks)	Serum cholesterol (mg/dl)	24.02	0.75	7.01	23.16	0.72	6.92	23.05	0.73	7.08
	Serum corticosterone (ng/ml)	2.9	0.078	6.04	2.7	0.075	6.18	2.5	0.070	6.29

Serum cholesterol levels increased in parallel with age, namely poultry production, being in control at all times the highest values in group B (23.15 ± 0.79 mg / dl at the beginning of laying, 24 , 02 ± 0.75 mg / dl during the plateau phase) compared to values measured in FR group that you were from 1.8 to 4.2% lower for the same period of control, while in samples blood taken from the batch CA, analytical values were intermediate, but closer to those in the FR group. This is normal, the known association of higher cholesterol values with state of metabolic stress, translated, as previously seen levels slightly increased in group B corticosterone, compared to only two other groups investigated (-0.8 ... - 1.16%).

Activity 4.3. Data acquisition – production intensity. Eggs sampling and preparation for analysis, separated on components (albumen, yolk)

There were the following parameters: average flock of birds, flock casualties, bird weight, food consumption, number of egg production, laying intensity, egg weight, egg weight with morphological abnormalities.

Casualties of the flock were due to adaptive effort to integrate biological material in a steady production, the resulting values are considered normal for the period of lay, in accordance to specified guide of hybrid technology. Analyzing the data in tab. 7 is observed that most losses are merged at the beginning and peak of laying, at this time the birds being used with technological equipment, the new food recipe, with new values on the surface density shared, not least with microclimate external factors, if the batch FR, which explains the higher values obtained in the latter group (5.2%-CA, 5.6% and 6.8%-B-FR).

Table 7

Dynamics fo fowl flocks, during 20-40 weeks age period

Fowl age (weeks)	Group B		Group CA		Group FR	
	Flock (hens)	Casualties (hens)	Flock (hens)	Casualties (hens)	Flock (hens)	Casualties (hens)
20	250	1	250	1	250	0
21	249	1	249	1	250	1
22	248	1	248	1	249	2
23	247	2	247	1	247	1
24	245	1	246	1	246	2
25	244	2	245	2	244	1
26	242	1	243	2	243	2
27	241	2	241	1	241	2
28	239	1	240	2	239	2
29	238	1	238	1	237	1
30	237	1	237	0	236	2
31	236	0	237	0	234	1
32	236	0	237	0	233	0
33	236	0	237	0	233	0
34	236	0	237	0	233	0
35	236	0	237	0	233	0
36	236	0	237	0	233	0
37	236	0	237	0	233	0
38	236	0	237	0	233	0
39	236	0	237	0	233	0
40	236	0	237	0	233	0
	Total hens:	14		13		17
	TOTAL %:	**5.60**		**5.20**		**6.80**

Table 8

Eggs yield dynamic and laying intensity, during 20-40 weeks age period

Fowl Age (weeks)	Group B			Group CA			Group FR		
	Flock	Eggs/week	% laying	Flock	Eggs/week	% laying	Flock	Eggs/week	% laying
20	250	789	45.11	250	760	43.45	250	720	41.17
21	249	913	52.37	249	879	50.44	250	825	47.13
22	248	1035	59.62	248	997	57.43	249	865	49.63
23	247	1128	65.24	247	1086	62.84	247	1029	59.54
24	245	1249	72.83	246	1208	70.15	246	1129	65.55
25	244	1364	79.84	245	1319	76.90	244	1135	66.47
26	242	1442	85.15	243	1395	82.02	243	1304	76.63
27	241	1536	91.03	241	1479	87.68	241	1401	83.07
28	239	1510	90.28	240	1461	86.96	239	1359	81.25
29	238	1483	89.04	238	1429	85.76	237	1230	74.13
30	237	1413	85.18	237	1361	82.05	236	1284	77.74
31	236	1391	84.23	237	1346	81.13	234	1149	70.12
32	236	1370	82.92	237	1325	79.87	233	1126	69.03
33	236	1308	79.15	237	1265	76.24	233	1178	72.23
34	236	1275	77.18	237	1233	74.34	233	1048	64.25
35	236	1269	76.83	237	1228	74.00	233	1144	70.12
36	236	1258	76.13	237	1217	73.33	233	1034	63.38
37	236	1246	75.42	237	1205	72.64	233	1123	68.83
38	236	1240	75.08	237	1200	72.32	233	1019	62.50
39	236	1237	74.87	237	1196	72.11	233	1017	62.33
40	236	1224	74.11	237	1184	71.38	233	1103	67.63

Numerical dynamics of birds flocks studied correlated with specific versions of each experimental study contributed to the achievement of productive performance different birds during the age of 20-40 weeks, as follows from the tab. 8.

Thus, the early laying (weeks 21), laying intensity showed higher values in group B(52.37%), intermediate in group CA (50.44%) and lowest for batch FR (47.13 %), which shows inadaptability hybrid system used to increase free-range, being created by artificial selection for growth in battery cages. The same hierarchy was maintained in peak laying (91.03%-B-CA 87.68%, 83.07%-FR) and in phase I of the plateau period (74.11%-B, 71, 38%-B, 67.63%-FR). Also, curve laying hens kept for experimental free-range version presented an atypical dynamic with sinusoidal oscillations, type "saw tooth" to the typical linear trend for this period due to stress of adapting to the environment (fig. 1).

For all lots, performances were below standard values characterizing the laying curve. This recommends putting into practice at farm level, increasing laying pullets from the age of one day to reach sexual maturity and then, during the production in the same farm, the same technology growth while respecting the specificity of each period of age, in terms of microclimate and nutritional requirements for the biological material used.

On shell quality minerals that egg production, the data tab. 9 are conclusive, they showing the proportion of abnormalities in the entire production of eggs.

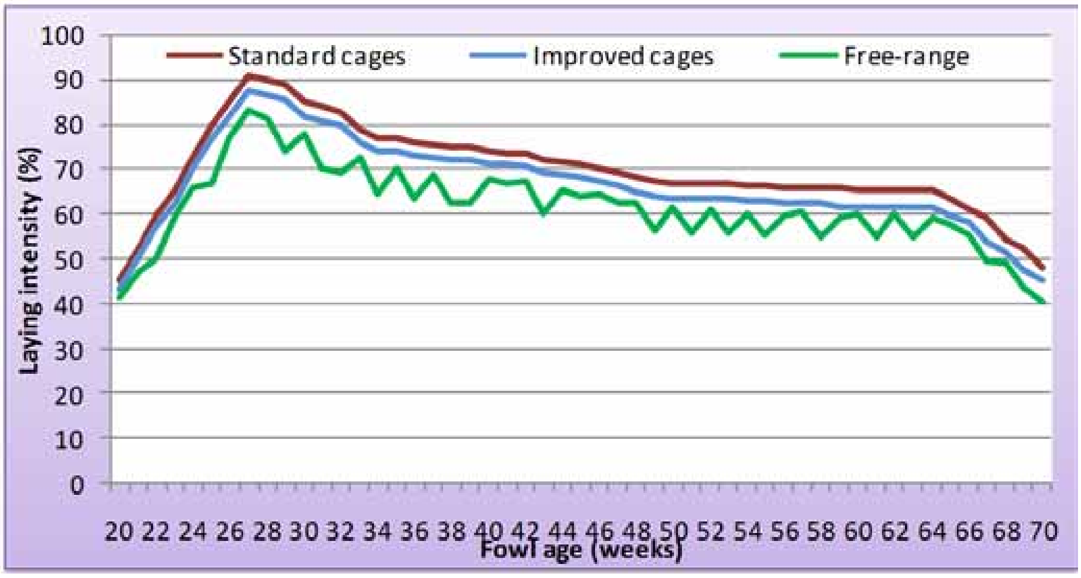

Fig. 1 – Laying curve during 20-70 weeks, in the hens from the 3 experimental versions

Table 9

Morphologic anomalies of the studied eggs, as related to the applied husbandry system

Control moment	Anomaly (%)	Exp. Group		
		B	CA	FR
Laying onset (21 weeks)	Eggs with malformed shell	0.83	0.72	0.69
	Eggs without yolk	0.05	0.04	0.05
	Eggs without shell	0.25	0.24	0.25
	Eggs with double yolk	0.12	0.11	0.08
	Eggs with cracked shell	0.89	0.81	0.72
	Total	**2.14**	**1.92**	**1.79**
Laying peak (27 weeks)	Eggs with malformed shell	0.41	0.35	0.38
	Eggs without yolk	0.02	0.02	0.01
	Eggs without shell	0.15	0.1	0.11
	Eggs with double yolk	0.03	0.03	0.04
	Eggs with cracked shell	0.62	0.68	0.61
	Total	**1.23**	**1.18**	**1.15**
Laying plateau stage II (40 weeks)	Eggs with malformed shell	0.56	0.42	0.45
	Eggs without yolk	0.04	0.03	0.04
	Eggs without shell	0.14	0.15	0.15
	Eggs with double yolk	0.04	0.03	0.04
	Eggs with cracked shell	0.58	0.61	0.63
	Total	**1.36**	**1.24**	**1.31**

We notice a higher percentage of abnormalities at the beginning of laying, when the unit accommodates reproductive still produces eggs with a rate increasing faster ovulation / oviposition, up to reach peak production. Thus, abnormalities percentage was highest in group B (2.14%), then as a group (1.92%) and the FR group (1.79%).It is noted that from total anomalies, the calcification of the shell aberations (malformed shell, shell absent) produced most of the losses so when oviposition or during subsequent handling of eggs (broken shell). The same hierarchy in the proportion of eggs remained non-compliance and lay on top, but with much lower values (max. 1.23%min B-group. 1.15%-FR group). In the plateau phase, the percentage of abnormalities began to rise again, due to fatigue accumulation reproductive, growth and egg volume, while the amount of shell deposited during laying remains relatively constant.

Activity 4.3 included the sampling at every time of control of 100 eggs, from which samples were composed of medium white, yolk and mix, which were then dried at 60 C and preserved by freezing, for tests analytical chemistry).

Activity 4.4. *Performing chemical analysis under Weende schematics (moisture, dry matter, ash, proteins, lipids). Calculation (determination) of eggs caloricity.*

For these parameters were carried out analysis / calculations in 20 repetitions. Assessing the amount of water and dry matter (DM) was carried out by the ovendrying method at 105 ° C (standard ISO 1442/1997). In order to measure the amount of minerals (ash) method was used calcination at 550 ° C (according to standard ISO936: 1998). Assessing the amount of fat was performed by using Soxhlet method directly on the extraction apparatus for quantitative separation of a mixture of substances by using an organic solvent, the model Velp Scientific - SER 148 (method specified by the manufacturer of the equipment, AOAC Official Methods ofAnalysis / 1990 and compatible with ISO 1443: 2008). For determination of total nitrogen materials (TNM) and the protein was used Kjeldahl method adapted to VelpScientifica system, composed of unit DK6 digestion and distillation unit UDK7(method specified by the manufacturer of equipment - 981:10, AOAC Official Methods of Analysis / 1990, compatible with ISO 937:2007). The content of nitrogen-free extractive substances (NFE) revealed by mathematical calculation, thedifference remaining after the fact, the SU (%) were low proportions of other chemical components, such as mineral or organic, such as: % NFE = Dry matter% – Ashes% – (Lipids% + Proteins%). Caloricity was calculated on the relation using the amount of energy emitted by burning 1 g of proteins, lipids and carbohydrates in the calorimetric bomb, according to the relation:

EB (Kcal/Kg) =5.70 Kcal x g proteins + 9.50 Kcal x g lipids + 4.2 Kcal x g NFE

For the early laying (21 weeks), chemical composition analysis (Table 10) have revealed relatively similar among samples from the three groups investigated, which resulted in minor responses in ANOVA test, the comparisons arithmetic mean of each triplet.

Table 10

Eggs chemical composition, as related to laying hens husbandry system, during laying onset (fowl age=21 weeks)

Egg compartment	Chemical constituants (%)	Group B (n=20)			Group CA (n=20)			Group FR (n=20)		
		$\overline{X}$	$\pm s_{\overline{x}}$	V%	$\overline{X}$	$\pm s_{\overline{x}}$	V%	$\overline{X}$	$\pm s_{\overline{x}}$	V%
Yolk	Water	56.84	1.45	12.05	56.92	1.44	11.93	57.01	1.41	11.70
	D.M.*	43.16	1.04	11.38	43.08	1.06	11.57	42.99	1.05	11.57
	Ashes	1.26	0.02	8.00	1.19	0.02	7.92	1.17	0.02	7.77
	T.N.M.*	13.52	0.26	9.22	13.44	0.26	9.11	13.28	0.26	9.11
	Lipids	25.61	0.40	7.45	25.42	0.40	7.46	25.39	0.39	7.31
	N.F.E.*	2.77	0.04	7.32	3.03	0.05	7.23	3.15	0.05	7.23
Albumen	Water	87.84	1.97	10.57	87.92	2.00	10.76	88.01	1.97	10.56
	D.M.	12.16	0.29	11.20	12.08	0.28	11.06	11.99	0.28	10.87
	Ashes	0.71	0.01	6.32	0.64	0.01	6.32	0.62	0.01	6.20
	T.N.M.	11.03	0.22	9.20	11.07	0.22	9.19	11.1	0.22	9.19
	Lipids	0.10	0.002	10.20	0.09	0.002	10.09	0.09	0.002	9.90
	N.F.E.	0.32	0.01	9.37	0.28	0.01	9.25	0.18	0.004	9.25
Whole egg	Water	73.96	0.98	6.24	74.08	0.99	6.34	74.11	0.98	6.23
	D.M.	26.04	0.34	6.23	25.92	0.35	6.34	25.89	0.35	6.33
	Ashes	1.16	0.02	8.05	1.12	0.02	7.95	1.1	0.02	7.81
	T.N.M.	12.35	0.19	7.35	12.42	0.20	7.49	12.45	0.20	7.48
	Lipids	10.18	0.16	7.47	10.03	0.16	7.37	9.95	0.15	7.24
	N.F.E.	2.35	0.04	8.74	2.35	0.04	8.65	2.39	0.04	8.48

* D.M. = dry matter; T.N.M.=total nitrogen matters; N.F.E.=nitrogen free extract

Eggs in FR group showed the highest water content (74.11 ± 0.98%), therefore the lowest in fat (9.95 ± 0.15%) than at the other extreme group (B) whose egg samples were poorest in water (73.96 ± 0.98%) and most dense in fat (10.18 ±0.16%). Egg protein content ranged between 12.35 ± 0.19% (group B) - 12.45 ±0.20% (FR group).

Typically, chemical composition values at the beginning of laying can not be considered conclusive, since birds are just beginning production, egg weight is highly variable, as well as number of egg production, and adaptation to new food recipe (21-5). Therefore more suggestive are the average values calculated from tests performed on samples collected in peak egg laying (Table 11).Thus, statistically significant difference (group B vs. CA vs CA group. FR) and significant distinct (group B vs. Consignment FR) for SU content of yolk and water, and fat content of the same component of egg (27.02 ± 0.44%-FR, 27.36 ± 0.39%and 27.61 ±-CA-B 0.41%), which shows the influence of rearing system on the chemical composition of eggs.

For the period of plateau, differences between groups were more subdued (Table12), with no statistical significance when different strings have been subject to particularly ANOVA test. However worth noting the trend of chemical constituents proportional situation, that poorer eggs in water and high-fat when produced in conventional batteries, the density of the largest and richest people in the water, that is quantitatively lower lipid to eggs free-range type. Perhaps this phenomenon is correlated with the metabolism of birds in response to microclimate factors that have been subject to reserve that tend to body fat in the abdominal area to free-range hens in the system and therefore eliminate triglycerides and other lipid components in a smaller proportion in eggs.

Table 11

Eggs chemical composition, as related to laying hens husbandry system, during laying peak (fowl age=27 weeks)

Egg compartment	Chemical constituants (%)	Group B (n=20)			Group CA (n=20)			Group FR (n=20)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Yolk	Water	55.72^{a}	1.33	11.30	56.09^{b}	1.27	10.72	56.42^{c}	1.46	12.25
	D.M.*	44.28^{c}	1.05	11.21	43.91^{b}	0.98	10.49	43.58^{a}	1.12	12.08
	Ashes	1.29	0.02	7.63	1.22	0.02	7.14	1.19	0.02	8.18
	T.N.M.*	13.71	0.26	8.82	13.57	0.24	8.26	13.32	0.27	9.51
	Lipids	27.61^{c}	0.41	7.06	27.36^{b}	0.39	6.70	27.02^{a}	0.44	7.65
	N.F.E.*	1.67	0.02	6.89	1.76	0.02	6.54	2.05	0.03	7.50
Albumen	Water	88.08	1.90	10.19	88.12	1.81	9.67	88.18	2.06	11.05
	D.M.	11.92	0.26	10.49	11.88	0.25	9.95	11.82	0.28	11.37
	Ashes	0.66	0.01	5.99	0.61	0.01	5.68	0.57	0.01	6.49
	T.N.M.	10.98	0.20	8.76	11.01	0.19	8.31	11.09	0.22	9.54
	Lipids	0.09	0.002	9.72	0.08	0.002	9.10	0.08	0.002	10.43
	N.F.E.	0.19	0.00	8.96	0.18	0.00	8.39	0.08	0.002	9.66
Whole egg	Water	74.02	0.96	6.12	74.15	0.90	5.72	74.22	1.03	6.56
	D.M.	25.98	0.33	6.03	25.85	0.31	5.73	25.78	0.36	6.57
	Ashes	1.18	0.02	7.54	1.17	0.02	7.16	1.15	0.02	8.17
	T.N.M.	12.49	0.19	7.12	12.53	0.18	6.76	12.55	0.21	7.76
	Lipids	10.23	0.15	6.99	10.20	0.14	6.64	10.08	0.16	7.58
	N.F.E.	2.08	0.04	8.19	1.95	0.03	7.77	2.00	0.04	8.88

ANOVA: ^{ab}different superscripts reveal significant statistical differences (p<0.05) between groups
^{ac}different superscripts reveal significant statistical differences (p<0.01) between groups
* D.M. = dry matter; T.N.M.=total nitrogen matters; N.F.E.=nitrogen free extract

Table 12

Eggs chemical composition, as related to laying hens husbandry system, during plateau-stage I (fowl age=40 weeks)

Egg compartment	Chemical constituants (%)	Group B (n=20)			Group CA (n=20)			Group FR (n=20)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Yolk	Water	55.93	1.39	11.77	56.14	1.46	12.24	56.45	1.49	12.50
	D.M.*	44.07	1.09	11.64	43.86	1.12	12.09	43.55	1.14	12.33
	Ashes	1.31	0.02	7.91	1.24	0.02	8.20	1.23	0.02	8.35
	T.N.M.*	13.91	0.27	9.17	13.68	0.29	10.07	13.54	0.30	10.38
	Lipids	28.03	0.44	7.36	27.98	0.45	7.65	27.67	0.46	7.81
	N.F.E.*	0.82	0.01	7.20	0.96	0.02	7.49	1.11	0.02	7.66
Albumen	Water	88.11	1.98	10.62	88.16	2.18	11.69	88.21	2.25	12.06
	D.M.	11.89	0.28	10.93	11.84	0.29	11.37	11.79	0.29	11.61
	Ashes	0.65	0.01	6.24	0.6	0.01	6.87	0.58	0.01	7.08
	T.N.M.	10.95	0.21	9.15	11	0.22	9.53	11.01	0.23	9.74
	Lipids	0.08	0.002	10.07	0.07	0.002	10.45	0.08	0.002	10.65
	N.F.E.	0.21	0.004	9.31	0.17	0.004	10.23	0.12	0.003	10.54
Whole egg	Water	74.08	0.99	6.34	74.19	1.09	6.96	74.26	1.13	7.16
	D.M.	25.92	0.35	6.30	25.81	0.38	6.95	25.74	0.39	7.17
	Ashes	1.17	0.02	7.86	1.15	0.02	8.65	1.17	0.02	8.92
	T.N.M.	12.58	0.20	7.44	12.55	0.21	7.75	12.52	0.21	7.92
	Lipids	10.23	0.16	7.29	10.20	0.16	7.58	10.08	0.17	7.74
	N.F.E.	1.94	0.04	8.54	1.91	0.04	9.39	1.97	0.04	9.69

* D.M. = dry matter; T.N.M.=total nitrogen matters; N.F.E.=nitrogen free extract

Depending on the chemical constituents identified analytically, eggs caloricity was calculated in the 3 moments of control, based on a linear trend of increase in raw power with age of birds, due to the size of the egg volume, thus increasing the amount of nutrients and excreted in the eggs (Table 13).

Table 13

Caloricity of the eggs, as related to laying hens husbandry system, during laying onset, peak and plateau-stage I

Control moment	Caloricity per egg compartment	Group B (n=20)			Group CA (n=20)			Group FR (n=20)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Laying onset (21 weeks)	Kcal /100 g yolk	331.99	7.10	10.09	330.82	6.92	9.87	330.13	6.61	9.45
	Kcal /100 g albumen	65.17	1.33	9.63	65.13	1.27	9.18	64.88	1.27	9.21
	Kcal /100 g egg mass	176.98	2.86	7.62	175.95	2.81	7.54	175.53	2.82	7.58
	Kcal//whole egg of 60 g	106.19	1.71	7.62	105.57	1.69	7.54	105.32	1.69	7.58
Laying peak (27 weeks)	Kcal /100 g yolk	347.46	7.43	10.09	344.66	7.21	9.87	341.22	6.83	9.45
	Kcal /100 g albumen	64.24	1.31	9.63	64.27	1.25	9.18	64.31	1.25	9.21
	Kcal /100 g egg mass	177.04	2.88	7.69	176.46	2.85	7.62	175.40	2.85	7.66
	Kcal//whole egg of 60 g	106.22	1.73	7.69	105.87	1.71	7.62	105.24	1.71	7.66
Plateau stage I (40 weeks)	Kcal /100 g yolk	349.02	7.50	10.15	347.82	7.27	9.87	344.71	6.94	9.51
	Kcal /100 g albumen	64.06	1.33	9.78	64.08	1.25	9.18	64.02	1.26	9.29
	Kcal /100 g egg mass	177.71	2.89	7.69	176.92	2.86	7.62	175.78	2.85	7.66
	Kcal//whole egg of 60 g	106.63	1.74	7.69	106.15	1.71	7.62	105.47	1.71	7.66

However, the most dietetic eggs were identified in FR group (105.32 Kcal / egg in early laying, 105.24 kcal / egg during laying peak and 105.47 kcal / egg in plateau) compared with group B (106.19 kcal / egg in early laying, 106.22 kcal / egg laying peak and 106.63kcal / egg plateau).

Activity 4.5. *Test for identification of eggs content in fatty acids and cholesterol (gas chromatography / liquid) and stress hormones.*

All tests were performed by 5 repetitions / sample / batch, according to the methodology outlined above.

Nutritional quality of lipids from eggs produced by birds reared in three different technological systems is given, in fact, by the spectrum of the fatty acid composition of fats, detailed for each moment of the laying curve (Table 14-16). Although the excreted lipid structure is approximately similar, being given the usage of the same mixed fodder to feed the three groups of birds, there are slight differences on the relationship between the three categories of fatty acids, relevant both biochemical and nutritionally.

Table 14

Fatty acids content of the eggs produced in different technological systems during laying onset (fowl age=21 weeks)

Fatty acids profile	M.U.	Group B		Group CA		Group FR	
		Yolk	Whole Egg	Yolk	Whole Egg	Yolk	Whole egg
Saturated FA:	g/100g	**9.587**	**3.115**	**9.483**	**2.978**	**9.362**	**2.95**
12:0	g/100g	0.008	0.003	0.010	0.002	0.008	0.002
14:0	g/100g	0.099	0.029	0.082	0.035	0.084	0.063
16:0	g/100g	6.789	2.226	6.639	2.194	6.615	2.172
18:0	g/100g	2.269	0.783	2.303	0.637	2.248	0.618
Monounsaturated FA:	g/100g	**11.701**	**3.704**	**11.404**	**3.581**	**11.281**	**3.516**
16:1	g/100g	0.863	0.183	0.857	0.179	0.847	0.163
18:1	g/100g	10.276	3.361	10.209	3.251	10.181	3.238
20:1	g/100g	0.010	0.002	0.020	0.001	0.014	0.001
22:1	g/100g	0.007	0.002	0.009	0.002	0.005	0.002
Polyunsaturated FA:	g/100g	**4.262**	**1.865**	**4.198**	**1.844**	**4.162**	**1.831**
18:2	g/100g	0.329	1.426	0.348	1.407	0.335	1.389
18:3	g/100g	0.087	0.037	0.109	0.032	0.102	0.029
20:4	g/100g	0.392	0.169	0.403	0.155	0.387	0.143
20:5 ω-3	g/100g	0.009	0.003	0.009	0.002	0.008	0.002
22:5 ω-3	g/100g	0.004	0.008	0.003	0.007	0.005	0.007
22:6 ω-3	g/100g	0.099	0.062	0.101	0.059	0.097	0.054
SFA:MUFA:PUFA		**2.25:2.75:1**	**1.67:1.99:1**	**2.26:2.72:1**	**1.61:1.94:1**	**2.25:2.71:1**	**1.61:1.92:1**

Table 15

Fatty acids content of the eggs produced in different technological systems during laying peak (fowl age=27 weeks)

Fatty acids profile	M.U.	Group B		Group CA		Group FR	
		Yolk	Whole Egg	Yolk	Whole Egg	Yolk	Whole egg
Saturated FA:	g/100g	**9.682**	**3.185**	**9.738**	**3.108**	**9.491**	**3.142**
12:0	g/100g	0.010	0.004	0.011	0.003	0.009	0.002
14:0	g/100g	0.121	0.031	0.095	0.028	0.089	0.051
16:0	g/100g	6.807	2.238	6.754	2.201	6.702	2.184
18:0	g/100g	2.280	0.801	2.319	0.646	2.261	0.631
Monounsaturated FA:	g/100g	**11.891**	**3.767**	**11.729**	**3.764**	**11.428**	**3.753**
16:1	g/100g	0.871	0.191	0.865	0.187	0.851	0.174
18:1	g/100g	10.298	3.395	10.245	3.316	1.209	3.291
20:1	g/100g	0.011	0.002	0.021	0.001	0.017	0.011
22:1	g/100g	0.008	0.003	0.011	0.002	0.009	0.004
Polyunsaturated FA:	g/100g	**4.302**	**1.917**	**4.287**	**1.919**	**4.204**	**1.944**
18:2	g/100g	0.341	1.432	0.358	1.442	0.355	1.439
18:3	g/100g	0.099	0.039	0.115	0.047	0.114	0.041
20:4	g/100g	0.405	0.175	0.412	0.185	0.392	0.176
20:5 ω-3	g/100g	0.009	0.003	0.010	0.010	0.008	0.009
22:5 ω-3	g/100g	0.004	0.009	0.005	0.007	0.005	0.008
22:6 ω-3	g/100g	0.101	0.069	0.112	0.072	0.104	0.070
SFA:MUFA:PUFA		**2.25:2.76:1**	**1.66:1.97:1**	**2.27:2.74:1**	**1.62:1.96:1**	**2.26:2.72:1**	**1.62:1.93:1**

Thus, the most valuable category of fatty acids, namely the polyunsaturated (PUFA) was best represented in the eggs from free-range system, while in the same eggs, the proportion of saturated AG was lower (-0.9 ... 5%) compared to eggs produced in other farming systems (conventional battery cages and improved). The ratio between the 3 categories of fatty acids (SFA: MUFA: PUFA) was 1.67:1.99:1 (group B), 1.61:1.94:1 (group CA), respectively 1.61:1.92:1 (FR group), at the beginning of laying, to keep roughly the same proportions as in other moments it was control (peak and plateau-phase I) , especially the absolute quantitative values were gradually increased as the amount of fat excreted in the egg became stronger in the chemical constituents of the egg.

The percentage difference between fatty acids quantity was restrained, between groups for each fatty acids group, at differences of 0.2-1.5%, as eggs yield became more uniform, quantitative and qualitative.

Table 16

Fatty acids content of the eggs produced in different technological systems during laying plateau – stage I (fowl age=40 weeks)

Fatty acids profile	M.U.	Group B		Group CA		Group FR	
		Yolk	Whole Egg	Yolk	Whole Egg	Yolk	Whole egg
Saturated FA:	g/100g	**9.764**	**3.229**	**9.808**	**3.149**	**9.581**	**3.166**
12:0	g/100g	0.011	0.005	0.025	0.004	0.015	0.003
14:0	g/100g	0.125	0.032	0.109	0.029	0.097	0.059
16:0	g/100g	6.817	2.241	6.778	2.215	6.719	2.207
18:0	g/100g	2.293	0.883	2.328	0.659	2.282	0.644
Monounsaturated FA:	g/100g	**11.964**	**3.792**	**11.763**	**3.822**	**11.557**	**3.782**
16:1	g/100g	0.889	0.201	0.879	0.192	0.865	0.183
18:1	g/100g	10.304	3.409	10.239	3.329	10.224	3.308
20:1	g/100g	0.012	0.003	0.028	0.002	0.022	0.012
22:1	g/100g	0.008	0.004	0.018	0.003	0.013	0.005
Polyunsaturated FA:	g/100g	**4.355**	**1.938**	**4.308**	**1.961**	**4.259**	**1.963**
18:2	g/100g	0.352	1.491	0.362	1.508	0.359	1.499
18:3	g/100g	0.101	0.052	0.121	0.054	0.119	0.046
20:4	g/100g	0.418	0.178	0.425	0.189	0.423	0.182
20:5 ω-3	g/100g	0.011	0.004	0.012	0.011	0.011	0.010
22:5 ω-3	g/100g	0.005	0.010	0.006	0.008	0.006	0.009
22:6 ω-3	g/100g	0.118	0.078	0.121	0.077	0.116	0.075
SFA:MUFA:PUFA		**2.24:2.75:1**	**1.67:1.96:1**	**2.28:2.73:1**	**1.61:1.95:1**	**2.25:2.71:1**	**1.61:1.93:1**

Also in the lipid composition of eggs ranges the cholesterol, as a nutrient enjoying great attention among human consumers, which desire animal products with low cholesterol. Like other lipid components, cholesterol showed an upward trend from the beginning of laying (213.16 ± 8.63 mg / egg - free range, 215.72 ± 8.76 mg / egg - improved cage, 220 , 69 ± 9.02 mg / egg - standard battery) to lay plateau phase I (217.31 ± 8.76 mg / egg - free range, 220.69 ± 8.80 mg / egg – improved cage; 230.36 ± 9.39 mg / egg - standard batteries). The differences were statistically insignificant, although the percentage achieved, for example, a difference from 3.5 to 6% between group B and group FR (Table 17).

On corticosteronee (indicator of stress hormone produced naturally by birds in the adrenal complex as adaptive response of normal maintenance conditions) excretion in eggs, the dynamics appeared similar, except that daily eliminated levels were much low (about 30% of serum) (Table 18), for example, the laying peak: 0.59 ± 0.019 ng / 100 g yolk and 0.41 ± 0.012 yolk ng / 100 g albumen in group B, 0, 55 ± 0.017 ng / 100 g yolk and 0.37 ±0.011 ng / 100 g albumen in group CA and 0.51 ± 0.015 ng / 100 g yolk and 0.34 ± 0.011 ng / 100 g albumen the FR group.

Table 17

Eggs cholesterol content, as related to hens husbandry system, during laying onset, peak and plateau-stage I

Control moment	Cholesterol per compartments	Group B (n=5)			Group CA (n=5)			Group FR (n=5)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Laying onset (21 weeks)	µg/100 g yolk	932.71	39.08	9.37	921.97	38.26	9.28	918.66	37.10	9.03
	µg/100 g albumen	*ND	-	-	ND	-	-	ND	-	-
	µg/100 g egg mass	367.82	15.03	9.14	359.53	14.60	9.08	355.27	14.38	9.05
	µg//whole egg of 60 g	220.692	9.02	9.14	215.72	8.76	9.08	213.16	8.63	9.05
Laying peak (27 weeks)	µg/100 g yolk	954.2	39.17	9.18	938.22	38.18	9.10	924.65	37.26	9.01
	µg/100 g albumen	ND	-	-	ND	-	-	ND	-	-
	µg/100 g egg mass	372.83	14.94	8.96	364.67	14.47	8.87	359.18	14.33	8.92
	µg//whole egg of 60 g	223.698	8.96	8.96	218.80	8.68	8.87	215.51	8.60	8.92
Plateau stage I (40 weeks)	µg/100 g yolk	963.81	39.96	9.27	947.61	38.56	9.10	936.58	38.03	9.08
	µg/100 g albumen	ND	-	-	ND	-	-	ND	-	-
	µg/100 g egg mass	383.94	15.64	9.11	367.82	14.67	8.92	362.18	14.59	9.01
	µg//whole egg of 60 g	230.364	9.39	9.11	220.69	8.80	8.92	217.31	8.76	9.01

*ND-undetectable

Table 18

Corticosteron content in eggs, as related to hens husbandry system During plateau-stage II and laying end periods

Control moment	Corticosteron in egg compounds	Group B (n=5)			Group CA (n=5)			Group FR (n=5)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Laying onset (21 weeks)	ng/ml yolk	0.51	0.020	8.95	0.49	0.019	8.84	0.49	0.019	8.89
	ng/ml albumen	0.38	0.012	7.32	0.35	0.012	7.35	0.33	0.011	7.41
Laying peak (27 weeks)	ng/ml yolk	0.59	0.019	7.02	0.55	0.017	6.89	0.51	0.015	6.72
	ng/ml albumen	0.41	0.012	6.57	0.37	0.011	6.61	0.34	0.010	6.57
Plateau-stage I (40 weeks)	ng/ml yolk	0.63	0.022	7.82	0.59	0.020	7.46	0.50	0.016	6.98
	ng/ml albumen	0.46	0.014	6.91	0.41	0.013	6.84	0.38	0.011	6.71

Data indicates a normal physiological adaptation response to all lots, but slightly more pronounced in birds reared at higher densities (superintensive system).

Objective 5 - Pilot Experiment: laying hens farming insuperintensive system (batteries of cages, conventional/improved) and free-range type-qualitative assessment of eggs production during laying plateau and ceasing moments

Activity 5.1. *Investigations on the physiological status of the studied fowl (haematological profile, blood chemistry).*

Protocolul experimental a urmărit acelaşi specific prezentat la activitatea 4.2.

For hematological parameters there were observed, similar as in the first moments ofcontrol, higher values in group B and groups that compared to FR for the amount of erythrocytes, suggesting a higher level of welfare in this experimental variant (improved cages); it ensured somewhat compromise between physiological needs on microclimate, manifestation of natural instincts and technological requirements. Lowest values were recorded in FR group (Table 19).

Also, in the FR group was found the highest level of white blood cells, due to daily exposure to the outside hall.

Table 19

Hematological parameters, as related to laying hens husbandry, during plateau-stage II and laying end

Control moment	Group	RBC (n=5) ($10^6/mm^3$)			PCV (n=5) (%)			MCV (n=5) (μm^3)			MCH (n=5) (pg)			MCHC (n=5) (g/100ml)			WBC (n=5) ($10^3/mm^3$)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Plateau-stage II (56 weeks)	**B**	2.71	0.09	7.05	30.76	0.48	3.47	113.51	2.63	5.18	48.29	1.16	5.37	42.54	1.01	5.28	22.81	0.73	7.16
	CA	3.02	0.11	8.19	31.63	0.73	5.13	104.74	3.07	6.56	42.65	0.92	4.83	40.72	1.04	5.70	27.08	1.02	8.41
	FR	2.32	0.09	8.83	30.24	0.75	5.54	130.34	4.13	7.08	51.61	1.35	5.84	39.59	1.15	6.47	28.48	1.14	8.97
Laying end (70 weeks)	**B**	2.97	0.10	7.24	32.11	0.51	3.57	108.11	2.57	5.32	51.10	1.26	5.51	47.26	1.15	5.42	23.74	0.79	7.43
	CA	3.08	0.12	8.57	32.53	0.78	5.37	105.62	3.24	6.87	44.75	0.99	4.97	42.37	1.12	5.93	27.64	1.08	8.71
	FR	2.35	0.10	9.67	30.68	0.70	5.07	130.55	4.24	7.26	53.90	1.43	5.92	41.29	1.22	6.60	29.72	1.31	9.83

Stress indicators have continued the same dynamics (Table 20), and they tended to return to higher values towards the end of laying, physical exhaustion occurs when the birds, after a strong production cycle: 3.2 ± 0.097 ng / ml plasma (B) - 3.0 ± 0.092ng / ml plasma (CA) - 2.8 ± 0.089 ng / ml plasma (FR). These levels decrease if stopping egg production and moulting occurs through a technology for recovering the metabolic supplies of birds.

Table 20

Blood serum biochemical parameters, as related to the hens husbandry system, during plateau-stage II and laying end

Control moment	Parameters	Group B (n=5)			Group CA (n=5)			Group FR (n=5)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Plateau stage II (56 weeks)	Serum cholesterol (mg/dl)	24.18	0.77	7.08	23.29	0.72	6.88	23.22	0.74	7.11
	Serum corticosterone (ng/ml)	3.0	0.083	6.18	2.9	0.083	6.39	2.7	0.077	6.41
Laying end (70 weeks)	Serum cholesterol (mg/dl)	24.36	0.78	7.12	23.57	0.72	6.83	23.41	0.74	7.09
	Serum corticosterone (ng/ml)	3.2	0.097	6.81	3.0	0.092	6.87	2.8	0.089	7.09

Serum cholesterol showed also increased values during the last two control periods, being approx. 3.4 to 4% higher in group B to FR and CA groups; there were not significant differences.

Activity 5.2. *Data acquisition – production intensity. Eggs sampling and preparation for analysis, separated on components (albumen, yolk)*

Experimental protocol followed the same specific presented in activity 4.3.

Towards the end of the plateau or laying end, there have occurred some of flock casualties caused by depletion of birds, due to intensive production rate. It is interesting to note that in this second period of the pilot study, most losses were found in groups accommodated in cages, whether conventional or modified. Thus, the initial losses were added with 4.66%(group B), 4.22% (group CA) and only 1.72% (FR group) (Table 21). Overall the experimental period, the best liveability rate was found in group FR (91.48%) and lowest was obtained in conventional farming systems in battery B (89.74%).

Table 21

Flock dynamics, during 41-70 weeks age interval

Fowl age (weeks)	Group B		Group CA		Group FR	
	Flock (hens)	Casualties (hens)	Flock (hens)	Casualties (hens)	Flock (hens)	Casualties (hens)
41	236	0	237	0	233	0
42	236	0	237	0	233	0
43	236	0	237	0	233	0
44	236	0	237	0	233	0
45	236	0	237	0	233	0
46	236	0	237	0	233	0
47	236	0	237	0	233	0
48	236	0	237	0	233	0
49	236	0	237	0	233	0
50	236	0	237	0	233	0
51	236	0	237	0	233	0
52	236	1	237	0	233	0
53	235	0	237	0	233	0
54	235	0	237	0	233	0
55	235	0	237	0	233	0
56	235	0	237	1	233	0
57	235	1	236	1	233	0
58	234	0	235	1	233	0
59	234	1	234	1	233	0
60	233	1	233	0	233	0
61	232	0	233	0	233	0
62	232	0	233	0	233	0
63	232	0	233	0	233	0
64	232	1	233	0	233	0
65	231	1	233	1	233	0
66	230	1	232	1	233	0
67	229	1	231	1	233	1
68	228	1	230	1	232	1
69	227	1	229	1	231	1
70	226	1	228	1	230	1
	Total hens:	11		10		4
	TOTAL %:	**4.66**		**4.22**		**1.72**

Eggs yield and laying intensity continued the downward trend, reaching 66.02% in group B weekly production, 62.86% group CA and only 59.42% if the FR group in phase II plateau (birds age of 56 weeks) (Table 22).

Table 22

Eggs yield dynamics and laying intensity, during 41-70 weeks age interval

Fowl Age (weeks)	Group B			Group CA			Group FR		
	Flock	Eggs/week	% laying	Flock	Eggs/week	% laying	Flock	Eggs/week	% laying
41	236	1220	73.82	237	1180	71.10	233	1084	66.44
42	236	1215	73.55	237	1175	70.84	233	1095	67.12
43	236	1193	72.19	237	1154	69.53	233	980	60.10
44	236	1181	71.5	237	1143	68.87	233	1064	65.25
45	236	1174	71.08	237	1136	68.46	233	1043	63.97
46	236	1160	70.22	237	1122	67.64	233	1045	64.08
47	236	1141	69.07	237	1104	66.53	233	1014	62.16
48	236	1132	68.53	237	1082	65.25	233	1020	62.54
49	236	1109	67.11	237	1060	63.90	233	911	55.87
50	236	1108	67.08	237	1060	63.87	233	998	61.22
51	236	1106	66.94	237	1057	63.73	233	909	55.73
52	236	1104	66.81	237	1055	63.61	233	994	60.97
53	235	1096	66.62	237	1052	63.43	233	905	55.46
54	235	1091	66.35	237	1048	63.17	233	974	59.71
55	235	1089	66.18	237	1045	63.01	233	899	55.09
56	235	1086	66.02	237	1043	62.86	233	969	59.42
57	235	1085	65.97	236	1038	62.81	233	982	60.20
58	234	1078	65.84	235	1031	62.69	233	894	54.81
59	234	1076	65.71	234	1014	61.93	233	965	59.14
60	233	1070	65.63	233	1009	61.86	233	977	59.89
61	232	1065	65.59	233	1008	61.82	233	891	54.60
62	232	1062	65.42	233	1006	61.66	233	974	59.70
63	232	1062	65.38	233	1005	61.62	233	888	54.43
64	232	1060	65.27	233	1003	61.52	233	958	58.74
65	231	1023	63.24	233	972	59.60	233	941	57.71
66	230	983	61.03	232	944	58.11	233	896	54.93
67	229	945	58.95	231	875	54.14	233	800	49.08
68	228	866	54.23	230	831	51.64	232	793	48.81
69	227	829	52.18	229	768	47.92	231	702	43.44
70	226	763	48.23	228	725	45.46	230	646	40.15

At the last control point (end of lay), average weekly production was 763 eggs (48.23%) in group B, 725 eggs (45.46%) in group CA and 646 eggs (40.15%) in FR group (Table 22) (fig. 1).

As approaching the moment of laying end the eggs morphological abnormalities have increased in frequency due to reduced thickness of the shell mineral and calcification errors, given the state of exhaustion of the reproductive system of birds (Table 23).

Thus, at the end of laying, eggs with morphologic non-compliances reached 2.24% birds raised in conventional batteries, 2.16% in the modified cages, and 2.08% in the free-range system.

Table 23

Morphological anomalies of the studied eggs, as related to the applied husbandry system

Control moment	Anomaly (%)	Experimental group		
		B	CA	FR
Laying plateau stage II (56 weeks)	Eggs with malformed shell	0.61	0.55	0.49
	Eggs without yolk	0.05	0.06	0.05
	Eggs without shell	0.15	0.17	0.15
	Eggs with double yolk	0.05	0.05	0.04
	Eggs with cracked shell	0.63	0.62	0.65
	Total	**1.49**	**1.45**	**1.38**
Laying end (70 weeks)	Eggs with malformed shell	0.65	0.62	0.54
	Eggs without yolk	0.11	0.1	0.12
	Eggs without shell	0.22	0.21	0.22
	Eggs with double yolk	0.06	0.06	0.05
	Eggs with cracked shell	1.2	1.17	1.15
	Total	**2.24**	**2.16**	**2.08**

Activity 5.3. *Performing chemical analysis under Weende schematics (moisture, dry matter, ash, proteins, lipids). Calculation (determination) of eggs caloricity.*
Experimental protocol followed the same specific presented in activity 4.4.

Towards the end of the plateau (56 weeks) results have revealed significant differences in water content between samples taken from FR group and the other two groups, housed in cages (Table 24).

Table 24

Eggs chemical composition, as related to laying hens husbandry system, during laying plateau – stage II (fowl age=56 weeks)

Egg compartment	Chemical constituants (%)	Group B (n=20)			Group CA (n=20)			Group FR (n=20)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Yolk	Water	56.08^{a}	1.45	12.21	56.21^{a}	1.49	12.53	56.52^{b}	1.49	12.42
	D.M.*	43.92^{b}	1.11	11.94	43.79^{b}	0.91	9.85	43.48^{a}	0.97	10.55
	Ashes	1.27	0.02	8.14	1.25	0.02	6.71	1.25	0.02	7.19
	T.N.M.*	14.03^{c}	0.28	9.40	13.81^{b}	0.28	9.64	13.74^{a}	0.28	9.56
	Lipids	28.11	0.45	7.58	28.06	0.37	6.25	27.83	0.39	6.69
	N.F.E.*	0.51	0.01	7.44	0.67	0.01	6.13	0.66	0.01	6.57
Albumen	Water	88.13	2.00	10.70	88.22	2.05	10.98	88.26	2.03	10.88
	D.M.	11.87	0.28	11.02	11.78	0.23	9.09	11.74	0.24	9.73
	Ashes	0.64	0.01	6.52	0.62	0.01	5.38	0.59	0.01	5.76
	T.N.M.	10.94	0.22	9.34	10.98	0.22	9.58	10.97	0.22	9.50
	Lipids	0.07	0.002	10.29	0.07	0.001	8.49	0.08	0.002	9.09
	N.F.E.	0.22	0.004	9.55	0.11	0.002	7.87	0.1	0.002	8.43
Whole egg	Water	74.13	1.02	6.49	74.25	1.05	6.66	74.31	1.04	6.60
	D.M.	25.87	0.36	6.51	25.75	0.29	5.37	25.69	0.31	5.75
	Ashes	1.16	0.02	7.92	1.15	0.02	8.12	1.16	0.02	8.06
	T.N.M.	12.61	0.20	7.65	12.59	0.21	7.85	12.57	0.21	7.78
	Lipids	10.28	0.16	7.57	10.25	0.14	6.24	10.14	0.14	6.68
	N.F.E.	1.82	0.03	8.86	1.76	0.03	9.08	1.82	0.03	9.01

ANOVA: $^{ab,\ bc}$different superscripts reveal significant statistical differences ($p<0.05$) between groups ^{ac} different superscripts reveal significant statistical differences ($p<0.01$) between groups

* D.M. = dry matter; T.N.M.=total nitrogen matters; N.F.E.=nitrogen free extract

Also, significant differences were found for total nitrogen content in the material (B vs.CA and FR vs. CA) and distinct significant from group B and FR for chemical constituents of the yolk (14.03 ± 0 ,28% in samples B vs 13.74 ±0.28% in samples FR).

Overall, the same trend observed in previous times, that eggs produced in free range system were slightly rich in water and low in fat, including in the end of laying (Table 25). There were registered again significant differences in water content and SU, between groupB and the other two groups studied, when we investigated the chemical composition of whole egg.

Table 25

Eggs chemical composition, as related to laying hens husbandry system, during laying end (fowl age=70 weeks)

Egg compartment	Chemical constituants (%)	Group B (n=20)			Group CA (n=20)			Group FR (n=20)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Yolk	Water	56.19[a]	1.67	14.01	56.27[a]	1.66	13.93	56.58[b]	1.69	14.07
	D.M.*	43.81[b]	1.13	12.19	43.73[b]	1.20	12.92	43.42[a]	1.03	11.18
	Ashes	1.24	0.02	8.31	1.24	0.02	7.71	1.23	0.02	7.13
	T.N.M.*	14.12	0.32	10.79	13.98	0.30	10.00	13.91	0.31	10.47
	Lipids	28.21	0.46	7.74	28.18	0.49	8.20	28.11	0.42	7.09
	N.F.E.*	0.24	0.00	7.59	0.33	0.00	7.04	0.17	0.00	7.38
Albumen	Water	88.19	2.29	12.28	88.22	2.13	11.39	88.24	2.23	11.92
	D.M.	11.81	0.28	11.25	11.78	0.28	11.18	11.76	0.25	9.98
	Ashes	0.63	0.01	6.66	0.63	0.01	6.62	0.62	0.01	6.70
	T.N.M.	11.01	0.25	10.72	11.04	0.23	9.94	11.04	0.21	9.19
	Lipids	0.06	0.001	10.51	0.06	0.001	11.14	0.05	0.001	9.63
	N.F.E.	0.11	0.002	9.75	0.05	0.001	9.04	0.05	0.001	9.46
Whole egg	Water	74.19[a]	1.17	7.45	74.32[b]	1.09	6.91	74.38[b]	1.14	7.24
	D.M.	25.81	0.36	6.65	25.68	0.36	6.61	25.62	0.36	6.69
	Ashes	1.17	0.02	9.09	1.15	0.02	9.63	1.17	0.02	9.44
	T.N.M.	12.66	0.24	8.78	12.64	0.25	9.30	12.63	0.24	9.10
	Lipids	10.36	0.17	7.72	10.31	0.16	7.16	10.25	0.16	7.51
	N.F.E.	1.62	0.03	10.16	1.58	0.03	10.10	1.57	0.03	10.20

ANOVA: [ab, bc]different superscripts reveal significant statistical differences ($p<0.05$) between groups

* D.M. = dry matter; T.N.M.=total nitrogen matters; N.F.E.=nitrogen free extract

Towards the end of laying, eggs caloricity continued to grow, regardless of production system used, so that, for an average egg weight (60g), it has emerged from 105.37 to 106.31 Kcal in the second phase the plateau, respectively from 105.58 to 106.43 Kcal during week 70 (end of lay), compared between free range and operating variants of conventional batteries (Table 26). Improved battery eggs showed intermediate values of gross energy.

Overall productive period is noted that the caloric value of eggs gradually increase, based on higher lipid content excreted through the yolk, while albumen gross energy decreases as volume increases in this component, as well as the water volume in egg also increases.

Table 26

Caloricity of the eggs, as related to laying hens husbandry system, during plateau-stage II and laying end periods

Control moment	Caloricity per egg compartment	Group B (n=20)			Group CA (n=20)			Group FR (n=20)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Plateau stage II (56 weeks)	Kcal /100 g yolk	349.16	7.55	10.21	348.10	7.34	9.96	345.48	7.11	9.72
	Kcal /100 g albumen	63.95	1.33	9.83	63.71	1.28	9.47	63.71	1.26	9.36
	Kcal /100 g egg mass	177.18	3.01	8.02	176.53	2.95	7.89	175.62	2.95	7.93
	Kcal//whole egg of 60 g	106.31	1.81	8.02	105.92	1.77	7.89	105.37	1.77	7.93
Laying end (70 weeks)	Kcal /100 g yolk	349.49	7.62	10.29	348.78	7.54	10.21	347.05	7.59	10.32
	Kcal /100 g albumen	63.79	1.35	9.97	63.71	1.33	9.84	63.61	1.35	10.04
	Kcal /100 g egg mass	177.39	3.10	8.24	176.63	3.06	8.19	175.96	3.09	8.29
	Kcal//whole egg of 60 g	106.43	1.86	8.24	105.98	1.84	8.19	105.58	1.85	8.29

Activity 5.4. *Test for identification of eggs content in fatty acids and cholesterol (gas chromatography / liquid) and stress hormones*
Experimental protocol followed the same specific presented in activity 4.5.

Analyzing the data in tab. 27 and 28, there is increased fatty acid content in all three categories, as they approach the end of laying, due to a concentration of lipids in the yolk.

Table 27

Fatty acids content of the eggs produced in different technological systems during laying plateau – stage II (fowl age=56 weeks)

Fatty acids Profile	M.U.	Group B		Group CA		Group FR	
		Yolk	Whole Egg	Yolk	Whole Egg	Yolk	Whole egg
Saturated FA:	g/100g	**9.901**	**3.351**	**9.981**	**3.224**	**9.767**	**3,206**
12:0	g/100g	0.014	0.006	0.027	0.005	0.021	0,004
14:0	g/100g	0.132	0.038	0.112	0.032	0.104	0,028
16:0	g/100g	6.925	2.257	6.782	2.234	6.754	2,219
18:0	g/100g	2.309	0.895	2.339	0.718	2.301	0,657
Monounsaturated FA:	g/100g	**12.208**	**3.928**	**11.944**	**3.895**	**11.774**	**3,870**
16:1	g/100g	0.907	0.209	0.891	0.203	0.872	0,196
18:1	g/100g	10.319	3.428	10.251	3.381	10.243	3,372
20:1	g/100g	0.015	0.004	0.032	0.003	0.029	0,003
22:1	g/100g	0.009	0.005	0.021	0.004	0.017	0,005
Polyunsaturated FA:	g/100g	**4.419**	**1.989**	**4.364**	**1.992**	**4.318**	**1,983**
18:2	g/100g	0.362	1.502	0.375	1.519	0.366	1,508
18:3	g/100g	0.124	0.064	0.136	0.068	0.128	0,061
20:4	g/100g	0.429	0.182	0.431	0.191	0.429	0,183
20:5 ω-3	g/100g	0.019	0.005	0.014	0.013	0.012	0,011
22:5 ω-3	g/100g	0.008	0.015	0.007	0.009	0.007	0,007
22:6 ω-3	g/100g	0.129	0.082	0.131	0.078	0.125	0,062
SFA:MUFA:PUFA		**2,24:2,76:1**	**1.68:1.97:1**	**2.29:2.74:1**	**1.62:1.96:1**	**2.26:2.73:1**	**1.62:1.95:1**

Consequences also notes, raising the ratio of SFA, MUFA and PUFA, from one moment to the next analysis. The best SFA: MUFA: PUFA was identified for the whole egg, in the FR group (1.62:1.95:1), close but not calculated for eggs produced by hens kept in improved cages (1.62:1.96:1).

The proportion of saturated fatty acids was reduced by 1.9 to 6% of eggs produced at the end of production in free range system compared to systems with improved battery or conventional batteries (Table 28).

Table 28

Fatty acids content of the eggs produced in different technological systems during laying end (fowl age=70 weeks)

Fatty acids Profile	M.U.	Group B		Group CA		Group FR	
		Yolk	Whole egg	Yolk	Whole Egg	Yolk	Whole egg
Saturated FA:	g/100g	**9.987**	**3.418**	**9.992**	**3.285**	**9.821**	**3,224**
12:0	g/100g	0.016	0.008	0.035	0.007	0.028	0,006
14:0	g/100g	0.141	0.042	0.129	0.038	0.116	0,032
16:0	g/100g	6.962	2.264	6.808	2.251	6.795	2,240
18:0	g/100g	2.318	0.918	2.349	0.887	2.304	0,818
Monounsaturated FA:	g/100g	**12.320**	**3.962**	**11.973**	**3.927**	**11.836**	**3,919**
16:1	g/100g	0.911	0.226	0.907	0.215	0.881	0,202
18:1	g/100g	10.340	3.441	10.281	0.384	10.265	0,378
20:1	g/100g	0.018	0.005	0.037	0.004	0.036	0,003
22:1	g/100g	0.010	0.007	0.025	0.005	0.021	0,005
Polyunsaturated FA:	g/100g	**4.483**	**2.025**	**4.381**	**2.018**	**4.349**	**2,007**
18:2	g/100g	0.385	1.538	0.394	1.545	0.402	1,527
18:3	g/100g	0.132	0.070	0.142	0.079	0.138	0,074
20:4	g/100g	0.447	0.195	0.458	0.201	0.456	0,198
20:5 ω-3	g/100g	0.022	0.006	0.018	0.012	0.015	0,012
22:5 ω-3	g/100g	0.010	0.017	0.009	0.014	0.009	0,013
22:6 ω-3	g/100g	0.135	0.089	0.141	0.083	0.136	0,069
SFA:MUFA:PUFA		**2,23:2,75:1**	**1.69:1.96:1**	**2.28:2.73:1**	**1.63:1.95:1**	**2.26:2.72:1**	**1.61:1.95:1**

Cholesterol content of eggs continued to trend upward, crossing the plateau phase II(220.97 ± 8.93 mg / egg - free range, 224.21 ± 9.07 mg / egg - improved cage, 233,56 ± 9.49 mg / egg - standard battery) and standing at 228.79 ± 4.40 mg / egg – free range, 233.77 ± 4.46 mg / egg - improved cage, 235.36 ± 4 , 55 mg / egg – standard batteries at the end of lay (70 weeks). Again, the percentage differences between group housed in standard battery and free-range system maintained in the range 2.8 to 5.7%, although differences were not statistically significant (Table 29).

Table 29

Cholesterol content in eggs, as related to hens husbandry system during plateau-stage II and laying end

Control moment	Cholesterol in egg compounds	Group B (n=20)			Group CA (n=20)			Group FR (n=20)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Plateau stage II (56 weeks)	µg/100 g yolk	970.63	40.41	9.31	958.19	39.21	9.15	944.72	38.74	9.17
	µg/100 g albumen	ND*	-	-	ND	-	-	ND	-	-
	µg/100 g egg mass	389.27	15.82	9.09	373.68	15.12	9.05	368.29	14.89	9.04
	µg/whole egg of 60 g	233.562	9.49	9.09	224.21	9.07	9.05	220.97	8.93	9.04
Laying end (70 weeks)	µg/100 g yolk	977.52	19.23	9.29	968.26	18.74	9.14	953.27	18.60	9.21
	µg/100 g albumen	ND	-	-	ND	-	-	ND	-	-
	µg/100 g egg mass	392.17	7.58	9.13	389.61	7.44	9.01	381.32	7.33	9.08
	µg/whole egg of 60 g	235.30	4.55	9.13	233.77	4.46	9.01	228.79	4.40	9.08

*ND=undetectable

At the end of production (Table 30), excreted corticosterone levels were also higher, depending on the dynamics corticosteronemy in serum, but keeping the same relative proportion of elimination (30-31%): 0.75 ± 0.027 ng / 100 g yolk and 0.48 ± 0.015 ng / 100 g albumen in group B, 0.69 ± 0.024 ng / 100 g yolk and 0.44 ± 0.014 ng / 100 g albumen in group CA, respectively 0.67 ± 0.022 ng / 100 g yolk and 0.41 ± 0.013 ng / 100 g albumen in the FR group.

Table 30

Corticosterone content in eggs, as related to hens husbandry system during plateau-stage II and laying end

Control moment	Corticosterone in egg compounds	Group B (n=5)			Group CA (n=5)			Group FR (n=5)		
		$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%	$\overline{X}$	$\pm s_{\bar{x}}$	V%
Plateau stage II (56 weeks)	ng/ml yolk	0.68	0.024	7.89	0.62	0.021	7.52	0.59	0.019	7.04
	ng/ml albumen	0.47	0.015	6.94	0.43	0.013	6.91	0.4	0.012	6.86
Laying end (70 weeks)	ng/ml yolk	0.75	0.027	7.95	0.69	0.024	7.71	0.67	0.022	7.31
	ng/ml albumen	0.48	0.015	6.98	0.44	0.014	6.93	0.41	0.013	6.96

Similar results were reported by other authors in international scientific data flow, given that birds rearing system does not significantly influence the response to stressful environmental factors, evidence is, at the same time, the high production level in superintensively or intensively husbandry systems. In fact, stress adaptation, for the genotype studied, was higher for free-range system, due to sudden fluctuations of microclimate factors, as confirmed by lower production levels and the lack of constancy in production.

Overall, the results suggest slightly higher dietary properties for eggs produced in free-range system (lower caloricity and proportion of cholesterol, better ratio between types of fatty acids with polyunsaturated favor of), compared with the conventional superintenisve and the alternative systems, but denies reports that some eggs produced in the superintensive system would be harmful to consumers, due to the presence of high concentrations of stress hormones in their composition (average values for corticosterone were similar between groups and followed the productive effort given by the dynamic curve of laying; it showed no differences with statistical significance and the data obtained are comparable to those reported in similar studies in other countries).

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