agriculture

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Effects of Genotype and Diet on Performance, Carcass Traits, and Blood Profiles of Slow-Growing Chicks Obtained by Crosses of Local Breed with Commercial Genotype

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Abstract: The effects of genotype and diet on growth performance, carcass traits and blood metabolites were investigated. The commercial Ross 308 (R) chickens genotype, a local Black Transylvanian Naked Neck (BTNN) breed, and their crosses were used in an 81-day study. A total of 720 one-d-old chicks were allotted into eight groups in a 4 × 2 factorial design with 4 genotypes: Rmale × Rfemale (R), BTNNmale × Rfemale (BTNN-R), BTNNmale × BTNNfemale (BTNN), Rmale × BTNNfemale (R-BTNN), and 2 diets: control and low-metabolisable energy (LME). Genotype affected performance parameters, namely body weight gain (BWG), feed intake (FI), energy intake (EI), feed conversion ratio (FCR), energy conversion ratio (ECR), and production efficiency factor (PEF), irrespective of growth phase ($p < 0.05$). Diet had no significant effect on overall BWG, EI, ECR and PEF, except that it increased FI and FCR. Genotype influenced the carcass and organ yields ($p < 0.05$), except bursa weight, while diet had no significant effect. Blood parameters (total cholesterol, triglycerides, glucose, albumin and phosphorus) were affected only by genotype ($p < 0.05$). In summary, results show that from the two crossbreedings obtained between R and BTNN genotypes, the BTNN-R growth performance and carcass traits were superior to R-BTNN, even though both have had a similar improved plasma response. Lowering the ME level did not significantly affect the BWG but increased FI and FCR, whereas the production index was similar regardless of the genotype. Based on the present results, we concluded that the BTNN-R crosses are the most suitable for use in alternative rearing systems for slow-growing chickens.

Keywords: blood response; carcass traits; low-metabolisable energy diet; performance; Ross 308; Black Transylvanian Naked Neck

Citation: Tudorache, M.; Custura, I.; Gheorghe, A.; Habeanu, M.; Lefter, N.A.; Pogurschi, E.N.; Popa, D.C. Effects of Genotype and Diet on Performance, Carcass Traits, and Blood Profiles of Slow-Growing Chicks Obtained by Crosses of Local Breed with Commercial Genotype. *Agriculture* **2022**, *12*, 1906. https://doi.org/10.3390/agriculture12111906

Academic Editor: Anna Milczarek

Received: 11 October 2022
Accepted: 10 November 2022
Published: 12 November 2022

1. Introduction

The global poultry meat production in 2021 was 135.4 million tons, which was 3.5% higher compared to 2019 and represented about 38% of the global meat production [1]. The consumer interest in poultry meat production increased due to many benefits issued from several essential and meaningful properties; it is affordable, a safe and healthy protein source, suitable for processing, and without any religious constraints concerning its consumption [2–4]. Due to this increased demand, the poultry sector has had to continuously modify its production strategies (i.e., genetic selection, economics, management, veterinary, and nutrition) to advance sustainability [5]. All these factors have contributed to a higher broiler growth rate and an increased production level emphasising quality and animal welfare [6]. Poultry production and meat quality are influenced by genotype, intensive selection [7,8], age, sex, production systems [9–14], and nutrition [15–18].

Nowadays, the slow-growing broiler breeders increased consumer interest by adding value to the meat qualities obtained using a specific rearing system adapted to the animal health, safety and welfare rules [19–21], although it is associated with increased production costs [22]. Previous studies found that slow-growing broilers adjust better to alternative rearing systems than fast-growing broilers [23] and have a superior meat quality corroborated with higher profitability [24–26].

An important and decisive limiting factor in poultry production is the feeding costs comprising about 70% of the total production costs; meanwhile, dietary energy sources represent the major cost (around 70%) of the total feed. Therefore, optimising the dietary energy level is an important tool to decrease production costs while considering performance and meat quality enhancement [27]. It was stated that increasing the dietary energy level improved the broilers' feed conversion ratio by decreasing feed intake [28,29], whereas using a higher energy level can alter carcass quality by increasing abdominal fat deposition [30]. Although there are several studies concerning the optimal dietary energy on broilers [31–33], there are not so many concerning slow-growing broilers [6,19,26,27].

On the other hand, it is well-known that the local breeds may not compete with the specialised lines regarding productivity, resources and economic efficiency. Still, they could be evaluated as dual-purpose breeds to supply niche markets and used in crossbreeding with commercial breeds [16]. In Romania, the dual-purpose breed Transylvanian Naked Neck (TNN) has gained attention due to its productive potentiality in alternative rearing systems linked with the efficient valorisation of feeds, adaptability to climatic conditions, and disease resistance [34]. This medium-sized breed of chicken reaches sexual maturity around 180–200 d of age and has different plumage colour varieties (black, red, barred, white). To reduce the significant decline in TNN livestock [35] as a consequence of economic inefficiency, and considering the current demands of consumers for slow-growing chickens with meat quality close to traditional chicken, the crossbreeding of this local breed with a commercial genotype could represent an opportunity to improve the sustainability and durability of rural areas [36]. So far, in Romania, there is no literature data available on the productivity of the local TNN breeds, or slow-growing chickens obtained by crossbreeding TNN breeds with a fast-growing commercial genotype, and their response to different dietary energy levels. Based on these considerations, we hypothesised, on one hand, that the TNN breed could provide the biological material to locally obtain slow-growing chickens for which Romania has a favourable perspective, and on the other hand, that genotype may have a different response in terms of productivity and blood metabolites to low dietary energy levels. Therefore, the present study was conducted to evaluate the effect of genotype (Ross 308, Black TNN breed and their crosses) and two dietary energy levels on growth performance, carcass characteristics, and blood profiles in chicks.

2. Materials and Methods

2.1. Ethical Statement

All trial procedures were approved by the Institutional Ethics Committee of the National Research Development Institute for Biology and Animal Nutrition (INCDBNA), Balotesti, Romania (Protocol no. 2277/14 April 2021). The chicks were managed and handled following the Romanian Law 43/2014 and EU principles of Directive 63/2010/EU regarding protecting the animals used for experimental and other scientific purposes [37].

2.2. Genotypes

The fast-growing genotype used was Ross-308 breeder (R), while the local Black TNN (BTNN) breed was used as the slow-growing genotype. Table 1 shows the parents' design used for crossbreeding to obtain the slow-growing chicks used in the present trial.

Table 1. Experimental parents design [1].

Mating Design	Abbreviations
R (Male) × R (Female): Rm × Rf	R
BTNN (Male) × R (Female): BTNNm × Rf	BTNN-R
BTNN (Male) × BTNN (Female): BTNNm × BTNNf	BTNN
R (Male) × BTNN (Female): Rm × BTNNf	R-BTNN

[1] The eggs incubation for each genotype was done during the same period at a local hatchery station (Dambovita county, Romania).

2.3. Chicks and Management

The trial was conducted for 81 days (d) from April to July 2021 at research Biobase INCDBNA-Balotesti (Ilfov county, Romania) in an environmentally controlled poultry house. A total of 720 d-old mixed-sex chicks (n = 180 chicks/genotype subdivided in two diets) were individually weighed and wing-tagged. The average weights of chicks from each genotype were 37.80 ± 0.32 g for R, 36.70 ± 0.35 g for BTNN-R, 36.50 ± 0.45 g BTNN, and 37.15 ± 0.16 g for R-BTNN. The chicks were raised under the same management conditions on a floor system in pens with wood-shaving litter. Each pen provided manual feeders and, in the first 4 d, the water was supplied by bell drinkers, and the rest of the trial by the nipple drinker line. The lighting program provided 23L:1D for the first 7 d and was gradually reduced to 16L:8D for the rest of the period, with a light intensity of about 20 lux. The immunisation protocol includes vaccinations against Marek's, Newcastle, Infectious Bursal, and Bronchitis. During the trial, birds had free access to water and feed. Feed was administrated in crumble or pelleted form according to the growth phase.

2.4. Experimental Design and Diets

Chicks were randomly allotted into eight groups (n = 90 chicks/group, six replicates of 15 chicks each) in a 4 × 2 factorial design with 4 genotypes, namely R, BTNN-R, BTNN, and R-BTNN, and 2 diets, namely control and low-metabolisable energy (LME). Based on chemical composition analyses of feed ingredients, the two-phase diets (1 to 28 d and 29 to 81 d) were formulated according to NRC [31] and Ross guide [38] to be isonitrogenous and with similar content of lysine and sulphury amino acids (Table 2). Compared to the C diet, the ME level was reduced by 100 kcal in the LME diet.

2.5. Chemical Analyses

The ingredients and diets samples were analysed in duplicate for their contents in dry matter (ISO 6496:2001), crude protein (ISO 5983-2:2009), crude fat (ISO 6492:2001), crude fibre (ISO 6865:2002), crude ash (ISO 2171:2010), minerals (calcium, ISO 6490-1:2006; phosphorus by photometric method), and amino acids profile by high-performance liquid chromatography method [39], according to EU Regulation no. 152/2009 [40].

2.6. Performance Variables

The performance parameters determined were body weight (BW) by individually weighing at d 1, 28 d and 81 d of age to calculate the body weight gain (BWG) for each growth phase (1 to 28 d; 29 to 81 d) and overall-phase (1 to 81 d). Feed intake (FI) per pen was recorded daily, and mortality was as well. Energy intake (EI), feed conversion ratio (FCR), and energy conversion ratio (ECR) were calculated for each growth phase and overall. The overall phase production efficiency factor (PEF) was calculated by the formula: livability (%) × BW (kg)/age (d) × FCR × 100.

Table 2. Ingredients and nutrient analyses of experimental diets.

Ingredients (% as-Fed)	1 to 28 d		29 to 81 d	
	C	LME	C	LME
Corn	54.22	55.72	55.54	56.90
Soybean meal	16.00	16.00	10.70	10.70
Rapeseed meal	5.00	5.00	8.00	8.00
Pea	15.00	15.00	18.00	18.00
Corn gluten meal	3.50	3.50	2.00	2.00
Vegetable oil	1.50	0	1.40	0
Monocalcium phosphate	1.70	1.70	1.50	1.50
Calcium carbonate	1.25	1.25	1.10	1.10
Salt	0.30	0.30	0.30	0.30
L-Lysine	0.22	0.22	0.20	0.20
DL-Methionine	0.21	0.21	0.20	0.20
Premix choline	0.10	0.10	0.10	0.10
Vitamin-mineral premix [1]	1.00	1.00	1.00	1.00
Analysed composition (%)				
Metabolisable energy (kcal/kg) [c]	3000	2900	3100	3000
Dry matter	89.53	88.90	89.87	89.75
Crude protein	20.10	20.14	18.10	18.06
Lysine	1.17	1.17	0.99	0.99
Digestible Lysine [c]	1.04	1.04	0.91	0.91
Methionine + Cysteine	0.89	0.89	0.80	0.80
Digestible Methionine + Cysteine [c]	0.80	0.80	0.72	0.72
Calcium	0.87	0.87	0.80	0.80
Available phosphorus [c]	0.44	0.44	0.39	0.40
Crude fat	3.39	2.78	4.96	4.50
Crude fibre	3.86	3.89	3.86	3.88
Crude ash	5.42	5.71	4.61	4.73

[1] Supplies per kg of diet: vitamin A—900,000 IU; vitamin D_3—200,000 IU; vitamin E—3000 IU; vitamin K_3—300 mg; vitamin B_1—200 mg; vitamin B_2—400 mg; vitamin B_3—2700 mg; vitamin B_5—1500 mg; vitamin B_6—300 mg; vitamin B_9—100 mg; vitamin B_{12}—1.8 mg; vitamin C—2000 mg; manganese—8000 mg; zinc—6000 mg; iron—8000 mg; cooper—500 mg; iodine—45 mg; selenium—18 mg; cobalt—25 mg; monensin sodium—60 mg (except for phase 29 to 81 d); antioxidant. [c] calculated values. Abbreviations: C, control diet; LME, low-metabolisable energy diet.

2.7. Slaughter Sampling

On d 81, after 12 h fasting, blood collection and slaughter measurements were performed with 12 chicks per group (6 male and 6 female) selected close to the average weight from each of the two diets. Blood was sampled from the wing vein using 23Gx3/4′-gauge needles into 4 mL heparinized tubes (Vacutest Kima, Italy).

Chicks were slaughtered by cervical dislocation, bled, and the carcasses were manually de-feathered and eviscerated. Carcasses were weighed, and carcass yield was calculated as % of BW at slaughter. Carcass cut-up parts, including breast, legs (with skin and bone), wings, back, abdominal fat, and internal organs, were removed, weighed, and their relative weights were expressed as % of BW at slaughter.

2.8. Blood Analyses

After blood centrifugation at 3000× *g* for 15 min at 4 °C (Centrifuge 5804R, Eppendorf AG, Hamburg, Germany), plasma was preserved in tubes at −20 °C until analysis. The plasma biochemical parameters, namely total cholesterol (TC), triglycerides (TG), glucose (Glu), total protein (TP), albumin (Alb), uric acid (UA), calcium (Ca), and inorganic phosphorus (IP), were determined by dry chemistry using specific reagent kits (Spotchem EZ SP-4430, Arkray Inc., Kyoto, Japan). The globulin (Glb) concentration was calculated as differences between TP and Alb.

2.9. Statistical Analysis

Data were analysed using the statistical software IBM SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) [41]. The normal data distribution was checked with Shapiro–Wilk's test. The data were analysed by the general linear model (GLM) procedure as a 4 × 2 factorial design using a two-way ANOVA following the model: Yijk = μ + Gi + Dj + (G × D)ij + eijk, where Yijk—dependent variables; μ—overall mean; Gi—effect of genotype (R, BTNN-R, BTNN, R-BTNN); Dj—effect of diet (control and low-metabolisable energy); (G × D)ij—interaction between genotype and diet; and eijk—residual error. The replicate pen was considered the experimental unit for growth performance and each chick's sample for carcass traits and blood variables. Results are shown as mean values with SEM (standard error of the mean). The significant mean differences were estimated using Tukey's posthoc test at $p < 0.05$.

3. Results

3.1. Growth Performance

Table 3 presents the productive performance of chicks as effect of genotype and diet. During the two growth phases and overall phase, all performance parameters (BWG, FI, EI, FCR and ECR) were significantly affected by the main factor genotype ($p < 0.05$). From 1 to 28 d phase, the BWG, FI, and EI differ significantly between all genotypes, with R having the highest values, followed by BTNN-R, R-BTNN crossbreds and BTNN. Regarding the FCR and ECR values, both were significantly increased in BTNN, intermediary in R-BTNN and BTNN-R crossbreds, and decreased in the R genotype. During 29 to 81 d, the higher BWG was achieved by R, the crossbreds R-BTNN and BTNN-R have similar values, and BTNN had the significant lowest value. The FI and EI significantly differed among all genotypes, with an increased intake in R and a decrease in BTNN. The R genotype obtained the most efficient FCR, while BTNN-R had the poorer value in this phase. The ECR were higher in BTNN-R and R-BTNN crossbreds than in R and BTNN genotypes. Overall phase (1 to 81 d) results showed that BWG were significantly higher in R, similar in BTNN-R and R-BTNN crossbreds, and lower in BTNN. At the same time, the FI and EI have a similar significant trend, with the highest values in R and the lowest in BTNN. The R and BTNN genotypes archived similar FCR and ECR, significantly different from the other genotypes. The PEF was significantly increased in R genotype, similar in BTNN-R and R-BTNN crossbreds, and reduced in the BTNN genotype.

Feeding the LME diet significantly influenced ($p < 0.05$) chicks' performance; during phase 1 to 28 d, BWG increased while EI, FCR, and ECR decreased; in phase 29 to 81 d, the FI, FCR and ECR increased whereas, in overall phase (1 to 81 d), no significant differences were found for BWG, EI, ECR and PEF, while FI and FCR significantly increased.

Genotype × diet interaction was found for FI, EI, FCR and ECR in 1 to 28 d and overall phases and for FI and EI in phases 29 to 81 d ($p < 0.05$).

Table 3. Effect of genotype and diet on growth performance [1] of chickens.

Item		1 to 28 d					29 to 81 d					1 to 81 d					
		BWG g	FI g	EI Kcal	FCR g/g	ECR Kcal/g	BWG g	FI g	EI Kcal	FCR g/g	ECR Kcal/g	BWG g	FI g	EI Kcal	FCR g/g	ECR Kcal/g	PEF
Genotype																	
R		1114 ^{a}	2117 ^{a}	6242 ^{a}	1.91 ^{b}	5.62 ^{b}	4605 ^{a}	14,574 ^{a}	44,446 ^{a}	3.18 ^{c}	9.68 ^{b}	5719 ^{a}	16,690 ^{a}	50,065 ^{a}	2.93 ^{c}	8.78 ^{c}	219 ^{a}
BTNN-R		595 ^{b}	1158 ^{b}	3417 ^{b}	1.95 ^{ab}	5.77 ^{ab}	2190 ^{b}	8283 ^{c}	25,235 ^{c}	3.79 ^{a}	11.54 ^{a}	2784 ^{b}	9442 ^{c}	28,296 ^{c}	3.39 ^{b}	10.17 ^{b}	97.1 ^{b}
BTNN		289 ^{d}	607 ^{d}	1794 ^{d}	2.21 ^{a}	6.58 ^{a}	898 ^{c}	3065 ^{d}	9354 ^{d}	3.42 ^{b}	10.44 ^{b}	1187 ^{c}	3672 ^{d}	11,025 ^{d}	3.10 ^{c}	9.31 ^{c}	42.7 ^{c}
R-BTNN		468 ^{c}	989 ^{c}	2916 ^{c}	2.12 ^{ab}	6.25 ^{ab}	2216 ^{b}	8702 ^{b}	26,536 ^{b}	3.55 ^{ab}	12.04 ^{a}	2685 ^{b}	9691 ^{b}	29,066 ^{b}	3.62 ^{a}	10.86 ^{a}	87.1 ^{b}
SEM		17.52	12.93	38.47	0.066	0.195	59.50	34.13	104.65	0.061	0.218	65.87	41.11	124.25	0.059	0.176	4.88
Diet																	
C		592 ^{b}	1215	3646 ^{a}	2.18 ^{a}	6.54 ^{a}	2531	8494 ^{b}	26,331	3.39 ^{b}	10.62 ^{b}	3123	9709 ^{b}	29,612	3.19 ^{b}	9.72	115
LME		640 ^{a}	1220	3538 ^{b}	1.91 ^{b}	5.54 ^{b}	2423	8815 ^{a}	26,455	3.57 ^{a}	11.22 ^{a}	3064	10,038 ^{a}	29,613	3.33 ^{a}	9.83	108
SEM		12.38	9.15	27.20	0.047	0.138	42.07	24.13	74.00	0.043	0.154	46.58	29.10	87.86	0.041	0.125	3.45
Genotype × Diet																	
R	C	1093	2068 ^{b}	6201 ^{a}	1.89 ^{b}	5.67 ^{bc}	4683	14,502 ^{a}	44,962 ^{a}	3.09	9.60	5776	16,570 ^{a}	50,542 ^{a}	2.87 ^{d}	8.75 ^{d}	226
	LME	1135	2167 ^{a}	6283 ^{a}	1.92 ^{b}	5.56 ^{bc}	4527	14,643 ^{a}	43,929 ^{b}	3.26	9.77	5662	16,810 ^{a}	49,588 ^{b}	2.98 ^{cd}	8.80 ^{cd}	211
BTNN-R	C	569	1147 ^{c}	3440 ^{b}	2.02 ^{b}	6.06 ^{bc}	2146	7702 ^{d}	23,875 ^{d}	3.60	11.15	2715	8849 ^{d}	26,988 ^{d}	3.27 ^{bc}	9.96 ^{bc}	99.1
	LME	620	1170 ^{c}	3393 ^{b}	1.89 ^{b}	5.48 ^{bc}	2234	8865 ^{b}	26,595 ^{c}	3.97	11.92	2854	10,035 ^{b}	29,603 ^{c}	3.52 ^{ab}	10.38 ^{ab}	95.0
BTNN	C	253	668 ^{e}	2005 ^{d}	2.65 ^{a}	7.95 ^{a}	949	3169 ^{e}	9825 ^{e}	3.34	10.36	1202	3837 ^{e}	11,704 ^{e}	3.19 ^{bcd}	9.74 ^{bcd}	42.9
	LME	324	546 ^{e}	1583 ^{e}	1.75 ^{b}	5.10 ^{c}	848	2961 ^{e}	8883 ^{f}	3.51	10.52	1172	3507 ^{f}	10,346 ^{f}	3.01 ^{cd}	8.87 ^{cd}	42.8
R-BTNN	C	454	979 ^{d}	2937 ^{c}	2.17 ^{b}	6.49 ^{b}	2348	8600 ^{b}	26,660 ^{c}	3.55	11.36	2801	9579 ^{c}	29,216 ^{c}	3.42 ^{b}	10.43 ^{ab}	91.1
	LME	483	998 ^{d}	2894 ^{c}	2.07 ^{b}	6.02 ^{bc}	2085	8804 ^{c}	26,412 ^{c}	3.54	12.70	2568	9802 ^{bc}	28,916 ^{c}	3.83 ^{a}	11.28 ^{a}	83.2
SEM		24.77	18.30	54.40	0.090	0.275	84.14	48.26	148.00	0.088	0.308	93.16	58.14	175.72	0.085	0.249	6.84
***p*-Value**																	
Genotype		0.0001	0.0001	0.0001	0.01	0.01	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Diet		0.011	NS	0.01	0.0001	0.0001	NS	0.0001	NS	0.009	0.01	NS	0.0001	NS	0.022	NS	NS
Genotype × Diet		NS	0.0001	0.001	0.0001	0.0001	NS	0.0001	0.0001	NS	NS	NS	0.0001	0.0001	0.014	0.015	NS

[1] Means of 90 chicks/group (15 chicks × 6 replicates each). Data were analysed as a 4 × 2 factorial design. $^{a–f}$ Means with different superscripts within a column differ ($p < 0.05$). Abbreviations: R = Ross 308; BTNN = Black Transylvanian Naked Neck; R = (Rm × Rf); BTNN-R = (BTNNm × Rf); BTNN = (BTNNm × BTNNf); R-BTNN = (Rm × BTNNf); C = control diet; LME = low-metabolisable energy diet; BWG = body weight gain; FI = feed intake; EI = energy intake; FCR = feed conversion ratio; ECR = energy conversion ratio; PEF = production efficiency factor; SEM = standard error of the mean; NS = not significant difference ($p > 0.05$).

3.2. Carcass Traits

Table 4 shows the effect of genotype and diet on carcass characteristics of chicks slaughtered at 81 d of age. Genotype significantly affects the carcass and organ yields ($p < 0.05$), except for the relative weight of bursa. The higher carcass yield was achieved by R, followed by BTNN-R, while R-BTNN and BTNN obtained similar carcass yield values. Breast yield was higher in the R genotype, similar in BTNN-R and R-BTNN crossbreds, and lower in BTNN. The R-BTNN had the lowest leg yield compared to the other genotypes. The R and R-BTNN genotypes have lower wings yield. Back yield was lower in BTNN-R compared to the other genotypes. The relative weights of heart and liver were higher in BTNN and R-BTNN genotypes. Gizzard weights were higher in BTNN and lowered in R genotype. Spleen was lower in R than the other genotype. Abdominal fat deposition was significantly higher in the R-BTNN and BTNN-R crossbreds than in parent genotypes R and BTNN.

Diet had no significant effect on carcass traits ($p > 0.05$). A genotype × diet interaction effect was found ($p < 0.05$) for breast and wing yields.

Table 4. Effect of genotype and diet on slaughter traits [1] of chickens at 81 d.

Item		Parameters (% of BW)										
		Carcass	**Breast**	**Legs**	**Wings**	**Back**	**Heart**	**Gizzard**	**Liver**	**Spleen**	**Bursa**	**Abd. Fat**
Genotype												
R		76.73 ^{a}	31.32 ^{a}	22.50 ^{a}	7.50 ^{b}	15.42 ^{a}	0.390 ^{b}	0.900 ^{c}	1.613 ^{b}	0.104 ^{b}	0.090	0.889 ^{c}
BTNN-R		68.97 ^{b}	21.18 ^{b}	22.96 ^{a}	9.94 ^{a}	14.88 ^{b}	0.395 ^{b}	1.515 ^{b}	1.722 ^{b}	0.155 ^{a}	0.108	1.693 ^{b}
BTNN		66.18 ^{c}	18.02 ^{c}	22.24 ^{a}	9.27 ^{a}	16.64 ^{a}	0.522 ^{a}	2.330 ^{a}	2.273 ^{a}	0.170 ^{a}	0.106	0.825 ^{c}
R-BTNN		66.41 ^{c}	21.74 ^{b}	21.01 ^{b}	7.64 ^{b}	16.65 ^{a}	0.475 ^{a}	1.293 ^{b}	1.978 ^{a}	0.158 ^{a}	0.101	2.556 ^{a}
SEM		0.367	0.397	0.331	0.183	0.461	0.016	0.074	0.057	0.012	0.008	0.218
Diet												
C		69.42	22.87	22.30	8.74	15.51	0.447	1.506	1.899	0.136	0.100	1.404
LME		69.73	23.26	22.05	8.43	16.29	0.444	1.513	1.894	0.157	0.102	1.577
SEM		0.260	0.281	0.234	0.130	0.326	0.012	0.052	0.040	0.008	0.006	0.154
Genotype × Diet												
R	C	76.32	30.31 ^{a}	22.46	7.71 ^{dc}	15.84	0.383	0.806	1.551	0.096	0.109	0.936
	LME	77.14	32.32 ^{a}	22.54	7.28 ^{dc}	14.99	0.395	0.993	1.674	0.111	0.070	0.842
BTNN-R	C	69.35	21.75 ^{b}	23.15	10.69 ^{a}	13.75	0.379	1.650	1.814	0.164	0.109	1.532
	LME	68.58	20.61 ^{bc}	22.77	9.17 ^{bc}	16.02	0.410	1.378	1.629	0.144	0.106	1.853
BTNN	C	66.39	18.29 ^{cd}	22.86	9.36 ^{b}	15.86	0.505	2.393	2.150	0.175	0.097	0.781
	LME	65.97	17.75 ^{d}	21.62	9.18 ^{bc}	17.43	0.537	2.267	2.395	0.164	0.114	0.868
R-BTNN	C	65.62	21.11 ^{b}	20.73	7.20 ^{dc}	16.57	0.517	1.174	2.081	0.109	0.086	2.365
	LME	67.20	22.37 ^{b}	21.27	8.08 ^{c}	16.72	0.434	1.411	1.875	0.207	0.117	2.747
SEM		0.184	0.199	0.166	0.092	0.230	0.008	0.037	0.029	0.006	0.004	0.109
***p*-Value**												
Genotype		0.0001	0.0001	0.001	0.0001	0.02	0.0001	0.0001	0.0001	0.002	NS	0.0001
Diet		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Genotype × Diet		NS	0.024	NS	0.001	NS	NS	NS	NS	NS	NS	NS

[1] Means of 12 chicks/group. Data were analysed as a 4 × 2 factorial design. $^{a–d}$ Means with different superscripts within a column differ ($p < 0.05$). Abbreviations: R = Ross 308; BTNN = Black Transylvanian Naked Neck; R = (Rm × Rf); BTNN-R = (BTNNm × Rf); BTNN = (BTNNm × BTNNf); R-BTNN = (Rm × BTNNf); C = control diet; LME = low-metabolisable energy diet; SEM = standard error of means; NS = not significant difference ($p > 0.05$).

3.3. Blood Profiles

The effect of genotype and diet on certain blood metabolites is given in Table 5. A significant effect of genotype was found for plasma parameters TC, TG, Glu, Alb, and IP ($p < 0.05$). TC concentration was higher in the R genotype, similar in BTNN-R and R-BTNN crossbreds, and lower in the BTNN genotype. The R genotype had the highest TG value than the other genotype, while the BTNN genotype had the lowest value but was not significantly different to BTNN-R and R-BTNN crossbreds. Glu level was higher in the

R genotype, while the other genotypes had similar values. The R genotype was found to have the highest Alb value. The IP concentration was higher in the R genotype, with no significant effect on the Ca/IP ratio. Dietary treatments did not alter plasma biochemical parameters ($p > 0.05$). No genotype × diet interaction was found.

Table 5. Effect of genotype and diet on plasma biochemical parameters [1] of chickens at 81 d.

Item		Energy			Protein				Mineral		
		TC mg/dL	TG mg/dL	Glu mg/dL	TP g/dL	Alb g/dL	Glb g/dL	UA mg/dL	Ca mg/dL	IP mg/dL	Ca/IP Ratio
Genotype											
R		126.2 ^{a}	56.25 ^{a}	231.6 ^{a}	3.76	2.28 ^{a}	1.49	4.79	7.79	4.47 ^{a}	1.74
BTNN-R		88.63 ^{b}	45.31 ^{bc}	216.2 ^{b}	3.49	2.06 ^{b}	1.43	4.53	7.58	4.22 ^{b}	1.80
BTNN		82.75 ^{c}	39.75 ^{c}	213.2 ^{b}	3.53	2.04 ^{b}	1.48	4.50	7.77	4.29 ^{b}	1.81
R-BTNN		96.13 ^{b}	51.81 ^{bc}	217.0 ^{b}	3.52	2.08 ^{b}	1.44	4.69	7.95	4.30 ^{b}	1.84
SEM		2.80	3.35	4.67	0.090	0.059	0.067	0.071	0.164	0.134	0.03
Diet											
C		95.94	45.06	214.2	3.55	2.10	1.45	4.54	7.77	4.41	1.76
LME		100.91	51.50	224.7	3.60	2.13	1.47	4.71	7.73	4.20	1.84
SEM		1.98	2.37	3.31	0.064	0.042	0.047	0.051	0.16	0.094	0.02
Genotype × Diet											
R	C	124.8	53.75	225.4	3.71	2.21	1.50	4.70	8.00	4.84	1.65
	LME	127.5	58.75	237.8	3.81	2.34	1.48	4.87	7.58	4.10	1.84
BTNN-R	C	84.62	40.75	210.1	3.43	2.08	1.35	4.38	7.50	4.30	1.74
	LME	92.62	49.87	222.2	3.55	2.05	1.50	4.68	7.65	4.15	1.84
BTNN	C	79.00	36.88	207.9	3.46	2.03	1.44	4.45	7.68	4.15	1.85
	LME	86.50	42.63	218.5	3.59	2.05	1.54	4.56	7.86	4.43	1.77
R-BTNN	C	95.25	48.88	213.5	3.58	2.06	1.53	4.65	7.90	4.38	1.80
	LME	97.00	54.75	220.5	3.46	2.09	1.38	4.73	7.82	4.23	1.84
SEM		3.97	4.75	6.62	0.128	0.08	0.10	0.10	0.23	0.19	0.04
***p*-Value**											
Genotype		0.0001	0.010	0.006	NS	0.033	NS	NS	NS	0.030	NS
Diet		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Genotype × Diet		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Means of 12 chicks/group; Data were analysed as a 4 × 2 factorial design. $^{a–c}$ Means with different superscripts within a column differ ($p < 0.05$). Abbreviations: R = Ross 308; BTNN = Black Transylvanian Naked Neck; R = (Rm × Rf); BTNN-R = (BTNNm × Rf); BTNN = (BTNNm × BTNNf); R-BTNN = (Rm × BTNNf); C = control diet; LME = low-metabolisable energy diet; TC = total cholesterol; TG = triglycerides; Glu = glucose; TP = total protein; Alb = albumin; Glb = globulin; UA = uric acid; Ca = calcium; IP = inorganic phosphorus; SEM = standard error of means; NS = not significant difference ($p > 0.05$).

4. Discussion

4.1. Growth Performance

The significant effect of genotype on growth performance (body weight gain, feed consumption, and feed conversion ratio) obtained between the genotypes and their crosses used in this study are supported by several reports [10,11,42–44]. Aggrey et al. [45] stated a positive genetic correlation exists between body weight gain and feed consumption. Several previous studies [46,47] reported that the increased feed intake in naked neck broilers than normally feathered birds is related to better thermo-regulatory efficiency and increased heat tolerance due to more exposed skin.

Our results revealed that the BWG was superior in chicks fed the LME diet only in phases 1 to 28 d with an improved FCR, while in the overall phase, the BWG had similar results, whereas FI and FCR were increased. These results partially agree with Attia et al. [26], who fed low-protein and low-protein-energy diets in Sasso slow-growing broilers and reported no effect on final weight, BWG, or FCR. The authors noticed that compared to the

control, these diets reduced feed and protein intakes, protein and metabolisable energy conversion ratios, as well as energy intake. Infante-Rodríguez et al. [29] found that energy level did not affect the BWG in broilers but reduced FI.

The significant genotype × diet interaction found for FI, EI, FCR, and ECR in overall phases suggests that these interactions were caused by the different responses of genotype to dietary treatments. For example, R genotype and BTNN-R and R-BTNN crosses showed the highest FI, FCR and ECR when fed the LME diet, while the BTNN genotype showed converse results.

4.2. Carcass Traits

The present results have shown a significant genotype effect on carcass yield, cut-up parts, organ yields, and abdominal fat, except the relative weight of the bursa. Cömert et al. [12] compared the genotype effect in fast- and slow-growing chicks (Ross 308 vs. Hubbard Red JA) in different rearing systems (organic vs. conventional). They reported higher live weight, carcass, breast, leg weights, and breast yield in Ross 308 and higher leg yield in Hubbard Red JA, whereas abdominal fat increased in the organic system. Isidahomen et al. [48] noticed a significant effect of genotype and sex, as well as an improved slaughter weight, carcass weight, and dressing yield in the naked neck vs. the frizzle chicken at 20 weeks. The current study revealed that the BTNN genotype had the lowest abdominal fat percentage of the genotypes. These results are in line with Isidahomen et al. [48], who reported variation in abdominal fat and fat percentage in normal feather, naked neck, and frizzled matured chickens' genotypes. Imasuen and Otoikhian [49] also found differences in abdominal fat from naked neck, frizzle birds, normal feathers and dominant black breeds of layer. Conversely, Fernandes et al. [10], when evaluating the carcass traits of different commercial genotypes (Ross, Cobb, Hubbard, and Arbor Acres), noticed no significant differences in carcass yield but a higher abdominal fat deposition in Ross, Cobb, and Hubbard than Arbor Acres. In addition, Mikulski et al. [15] reported lower breast and thigh muscle yield and a higher abdominal fat content in slower- vs. fast-growing Hubbard chickens.

Our results show that for the present genotypes studied, dietary LME level did not affect the carcass yield, the main carcass cuts traits, abdominal fat or relative organ weights. Similar findings were reported previously when fed different dietary energy levels close to the level used in our study by Copat et al. [50] in free range-broiler chicks, by Abouelezz et al. [27] in slow-growing Chinese yellow chickens, or by other reports on broilers [29,51,52]. Recently, Attia et al. [26] evaluated the low-protein-energy diet and supplementation with two phytases (*Escherichia coli* and *Aspergillus niger*) in Sasso slow-growing broilers for 64 d. These authors reported no effects on carcass traits, only a higher abdominal fat deposition when fed a low-protein-energy diet supplemented with *Aspergillus niger* than an unsupplemented diet.

Genotype × diet interaction was significant only for breast and wing yields, which suggests that these interactions were caused by the different responses of genotype to dietary treatments. For example, R and R-BTNN genotypes showed the highest breast yield of the genotypes when fed the LME diet, and lower wing yield irrespective of diet. The BTNN-R crossbred had higher wings yield when fed the C diet. The BTNN genotype showed the lowest breast yield in both diets.

4.3. Blood Profiles

Several studies [53–56] suggested that biochemical parameters in chickens could be affected by different factors, i.e., genotype, sex, nutrition, management and stress. Blood metabolites, such as total cholesterol and lipoprotein fractions, triglyceride, glucose, and protein concentrations, are important markers directly related to health [57] and meat quality [58]. The average cholesterol range in chickens is between 90–210 mg/dL [59]. Our study results found cholesterol levels of the R genotype to be in these ranges, but the cholesterol levels of the other genotypes were below. These results suggest that cholesterol

concentration can be decreased in slow-growing chickens depending on the crossing [47]. According to Griffin et al. [60] and Whitehead and Griffin [61], triglycerides are considered indices of total body fat. In the present study, the BTNN genotype had the lowest triglycerides level and the lower abdominal fat percentage. Similar findings were reported by Zein-El-Dein et al. [62] and Patra et al. [47] in coloured naked neck broilers compared with normally feathered broilers. These authors explain that the better heat tolerance of the naked neck can also be linked to its lower triglyceride level, allowing better heat dissipation. Adedeji et al. [63] also reported that the naked neck genotype displayed the lowest cholesterol, low-density lipoprotein, triglycerides, and abdominal fat amount, while the kuroiler naked neck crossbred chickens had the highest value of high-density lipoprotein. Sarica et al. [64], comparing different slow-growing chicken genotypes, found that lower blood triglyceride levels were associated with lower total protein levels. Our results showed that plasma total protein concentration was comparable to the normal reference of 40 mg/dL [55], but no significant effect of genotype used was noticed. Although the plasma total protein was not affected, the present results showed an increase in albumin and glucose concentrations in the R genotype compared to the others. However, these partially agree with previous studies [64,65], who stated that there is a link between the decrease of blood triglyceride and total protein and the increase of blood glucose attributed to gluconeogenesis.

Reducing the dietary energy level did not significantly change chicks' plasma blood metabolite response at 81 d of age. This is partially consistent with Attia et al. [26], who found no effect of decreasing the level of dietary protein or energy and protein on blood biochemistry, only an increase in plasma albumin with no changes in total protein content. In contrast, other research [66,67] found an increased plasma uric acid and reduced plasma concentrations of cholesterol and triglyceride to be an effect of reducing dietary energy in broilers.

5. Conclusions

The present study highlighted a significant effect of chickens' genotype on performance, carcass parameters, and some plasma metabolites response. Results show that from the two crossbreedings obtained between R and BTNN genotypes, the BTNN-R growth performance and carcass traits were superior to R-BTNN, even though both have had a similar improved plasma response. The use of LME diets did not significantly affect the BWG but increased FI and FCR, whereas the production index was similar regardless of the genotype used. Based on the present results, we concluded that the BTNN-R crosses are the most suitable for use in alternative rearing systems for slow-growing chickens. Further in-depth meat quality analysis investigations are still needed to support these findings.

Author Contributions: Conceptualisation, M.T., I.C. and A.G.; methodology, A.G., M.H. and N.A.L.; software, A.G.; validation, M.T., I.C. and A.G.; formal analysis, N.A.L., E.N.P. and D.C.P.; investigation, N.A.L. and A.G.; resources, M.T. and I.C.; data curation, A.G. and N.A.L.; writing—original draft preparation, A.G. and M.T.; writing—review and editing, A.G.; visualisation, all authors; project administration, M.T.; funding acquisition, M.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Romanian Ministry of Agriculture and Rural Development (grant ADER 8.1.9/2019), and supported thought the Ministry of Research, Innovation, and Digitalization (grant PFE 8/2021).

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the National Research Development Institute for Biology and Animal Nutrition, Balotesti (Protocol no. 2277/14 April 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Ioana Trifu and Vasile Birlogeanu for their technical support during the trial.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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