Rearing broilers as mixed or single-sex: relevance to performance, coefficient of variation, and flock uniformity

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ABSTRACT With known variation in performance between male and female broilers and the fact that sourcing single-sex birds for use in research is becoming increasingly difficult, it becomes important to determine the effect of rearing method with male and female broilers on between-pen variation and body weight (**BW**) uniformity. We evaluated the performance response of broilers reared as single or mixed-sex to standard and reduced crude protein (**CP**) diets. The study was designed as a 2 × 3 factorial arrangement of treatments consisting of 672 Cobb-500 broilers assigned to 48 floor pens with 6 treatments, 8 replicates, and 14 birds per pen. The factors were rearing method (male single-sex, female single-sex, or equally mixed-sex) and dietary CP level (standard or reduced). For the overall period of the trial (d 0−35) there was a significant effect ($P < 0.001$) of rearing method and CP level on feed intake (**FI**) and feed conversion ratio (**FCR**). There was also a significant interaction between rearing method and CP level for BWG during d 0 to 35 ($P < 0.01$). There was a significant interaction between CP level and sex on d 34 BW ($P < 0.01$) where the reduced CP diet decreased the BW of both males and females, but to a greater extent the BW of the female birds. Dietary CP level had a significant effect on relative breast and drumstick weights with birds fed the reduced CP diet having significantly lower breast weights ($P < 0.001$) and higher drumstick weights ($P < 0.01$).This study suggests that male and female broilers have different CP requirements, and rearing birds as equally mixed-sex results in the lowest CV% for performance parameters and best BW uniformity compared to single-sex birds. Furthermore, when low CP diets are fed to broilers, they will prioritize the growth of more important body parts such as the legs.

Key words: performance, rearing method, crude protein requirements, uniformity, carcass part weight

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INTRODUCTION

It has been widely accepted that male and female broilers differ in their growth performance (Young et al., 2001; Kidd et al., 2005; Lopez et al., 2011; Shafey et al., 2013; Benyi et al., 2015; Da Costa et al., 2017; Madilindi et al., 2018). However, research in the past has been performed on mainly male broilers due to easy access of single-sex birds for use in experiments and the reduced variation they provide (Gous, 2017). Recently, it has become increasingly difficult to source single-sex birds for use in experiments due to the fact that feather sexing is not always possible under certain circumstances. Ross 308 still has feather sexable birds in some regions, but changes in breeder genetics will make it more difficult to source feather sexable birds in the near future. This means researchers have to use mixed-sex broilers along with alternative sexing methods. Despite this, little research has been done to determine which rearing method (single-sex or equally mixed-sex) will result in the best uniformity within each experimental unit that is, pens/cages as well as the lowest between-pen variation. The benefit in the overall performance of birds reared as single or mixed-sex is contradictory. For example, some reports showed no benefits of sex separation on BWG (Hess et al., 1960; Lang et al., 1960; Lamoreux and Proudfoot, 1969) whilst others showed a clear benefit of single-sex rearing on performance and uniformity (Deaton et al., 1973; Gehle et al., 1974; de Albuquerque et al., 2006). More recently, Da Costa et al. (2017) reported that males benefited from mixed-sex rearing in terms of body weight gain (**BWG**) whilst females performed better reared as single-sex which is what we expect from our results. Indeed, most of the literature performed to evaluate the effect of rearing method on flock uniformity was done using less selected chicken breeds and it, therefore, becomes important to make the evaluation using broilers from modern genetic lines. It still remains true that due to the average faster growth

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rate of male broilers, there is generally poorer uniformity in mixed-sex flocks (Gous, 2017). Therefore, we hypothesized that single-sex pens will have better performance CV% and better BW uniformity compared to equally mixed-sex pens.

In our experiment, we used 2 different dietary crude protein (**CP**) levels (reduced CP and standard CP) to detect variation in performance between the different treatments. Wijtten et al. (2004) reported that responses of female broilers to increase levels of dietary protein were of a lower magnitude than those of male broilers. Hernandez et al. (2012) also reported that diets containing reduced CP negatively impacted the performance of male broiler but not females. From this data we can predict that in our experiment the performance responses between male and female broilers will be different when fed reduced CP diet. The last objective of our experiment is to determine the effect of sex and CP level on relative carcass part and fat-pad weights. Van Harn et al. (2019), reported that broilers fed diets low in CP had lower relative breast weights but higher relative leg weights and Chrystal et al. (2020) reported a linear increase in relative fat-pad weights as the CP % of the diet decreased. A higher proportion of abdominal fat was also reported in female broilers compared to males (Madilindi et al., 2018). There are conflicting views on the effect of sex on breast weights as Lopez et al. (2011) reported that male broilers had a lower relative breast meat yield compared to females, whereas Siaga et al. (2017) and Shafey et al. (2013) reported no significant effect of sex on breast weight. The current experiment might help to clarify the effect of sex on relative breast weight.

MATERIALS AND METHODS

Ethics Statement

This experiment was approved by the Animal Ethics Committee of the University of New England (Approval No. AEC20-004). All broiler management procedures including health care, husbandry and use of laboratory animals fulfilled the requirements of the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013).

Experimental Design and Bird Management

Day-old birds (mixed-sex Cobb-500 broiler chickens) were obtained from Baiada hatchery in Tamworth, NSW, Australia. Upon arrival, all birds were weighed, vent sexed, and allocated to floor pens according to treatments. A 2 × 3 factorial arrangement of treatments were performed with 672 birds assigned to 48 floor pens with 8 replicates per treatment, and 14 birds per replicate pen. The pen area minus feeder space was 1.05 m^2 and the stocking density at d 35 was 27.5 kg/m^2. The factors were rearing method (male single-sex, female single-sex, or equally mixed-sex i.e., 7 males and 7 females) and dietary CP level (standard or reduced). All the pens were located in the same environmentally controlled facility, equipped with feeders, and cup drinkers. The birds had ad libitum access to feed and water. The lighting, relative humidity and temperature were maintained following Cobb-500 guidelines (Cobb500, 2018).

Diets and Bird Performance

The ingredients and nutrient composition of the diets used in this experiment are shown in Table 1. The diets were formulated based on the inclusion of wheat and soybean meal. The standard crude protein (**SCP**) diets were formulated to meet the CP levels in the Cobb 500 guidelines (Cobb500, 2018) (starter 22%, grower 20%, and finisher 19%) and the RCP diets were formulated to have 2% less CP compared to the SCP diets (starter 20%, grower 18% and finisher 17%). The nitrogen content of the diets was analyzed using a combustion analyser (Leco model FP-2000 N analyser, Leco Corp., St. Joseph, MI) and the analyzed CP content of the diets is shown in Table 1. The RCP diet was formulated with additional L-valine, L-isoleucine, and L-arginine, which were added to the diets to ensure the amino acid levels in both treatments were the same. The amino acid content of the two finisher diets (SCP and RCP) was analyzed by the Australian Proteome Analysis Facility and is shown in Table 2. Phytase was included in all treatments at 0.01% (Quantum Blue, AB vista Feed Ingredients, Marlborough, UK). Titanium dioxide was added as an indigestible marker at 0.5% in the grower and finisher diets for both the SCP and RCP treatments. Birds were fed in starter (d − 10), grower (d 11−24), and finisher (d 25−35) phases. Both the birds and feed were weighed on arrival and on d 10, 24, and 35, and mortality was recorded daily. Feed intake (**FI**), BWG, and mortality adjusted feed conversion ratio (**FCR**) were calculated for all phases.

Carcass Parameter Measurements

Two birds from each of the single-sex pens were randomly selected and weighed on d 35. These birds were then electrically stunned and euthanized by cervical dislocation. Abdominal fat (considered as the fat from the proventriculus surrounding the cloaca and adjacent to the abdominal muscle) was taken out and weighed. Abdominal fat was then expressed as a percentage of live BW. All sampled birds were also processed for breast (skinless and boneless), thigh (with skin and bone), and drumstick weights which were expressed as proportion of live BW.

Body Weight Uniformity and CV% Calculations

The BW, FI, and FCR per pen within each treatment group were calculated to determine the CV% of the performance parameters according to the following equation:

$$CV\% = \left(SD_p/\overline{x}_p\right) \times 100. \quad (1)$$

Table 1. Composition and nutritional content of the experimental diets.

Item	Starter (d 0 to 10)		Grower (d 11 to 24)		Finisher (d 25 to 35)	
	SCP	RCP	SCP	RCP	SCP	RCP
Ingredients (DM basis), %						
Wheat	53.2	58.0	42.6	52.2	41.6	48.8
Soybean meal	25.6	21.0	26.4	25.0	22.2	15.1
Sorghum	15.0	15.0	25.0	16.9	30.0	30.0
Canola oil	1.37	0.94	2.51	1.19	2.90	2.02
Limestone (fine)	1.18	1.19	1.08	1.13	1.02	1.04
Dical Phos 18P/21Ca	0.89	0.97	0.70	0.75	0.58	0.62
Sodium bicarbonate	0.32	0.45	0.02	0.41	0.15	0.44
Titanium dioxide	-	-	0.50	0.50	0.50	0.50
L-lysine HCl 78.4	0.54	0.68	0.27	0.54	0.29	0.46
DL-methionine	0.39	0.42	0.27	0.33	0.23	0.27
L-Arginine	0.53	0.33	-	0.22	-	0.16
L-threonine	0.35	0.27	0.08	0.20	0.13	0.16
L-Valine	0.16	0.24	-	0.16	-	0.10
L-Isoleucine	0.12	0.20	-	0.14	-	0.05
NaCl	0.110	0.040	0.273	0.003	0.138	0.007
Trace mineral premix[1]	0.110	0.110	0.110	0.110	0.110	0.110
Vitamin premix[2]	0.085	0.085	0.085	0.085	0.085	0.085
Choline Cl 70%	0.048	0.080	0.053	0.080	0.069	0.090
Phytase	0.010	0.010	0.010	0.010	0.010	0.010
Xylanase	0.005	0.005	0.005	0.005	0.005	0.005
Nutrients, %						
AMEn, kcal/kg	3,005	3,009	3,075	3,075	3,147	3,150
Crude protein (calculated)	21.6	19.7	19.7	17.7	18.6	16.7
Crude protein (analyzed)	21.2	19.5	19.3	17.0	18.4	16.1
Crude fat	3.48	3.08	4.67	3.41	5.11	4.26
Crude fiber	2.49	2.42	2.51	2.37	2.45	2.34
Dig. Arg	1.62	1.31	1.12	1.12	1.03	1.02
Dig. Lys	1.28	1.28	1.08	1.08	0.995	0.97
Dig. Met	0.65	0.66	0.54	0.55	0.48	0.49
Dig. Ile	0.87	0.87	0.77	0.75	0.70	0.64
Dig. Val	1.01	1.01	0.88	0.86	0.81	0.78
Calcium	0.90	0.91	0.82	0.83	0.76	0.76
Av. P	0.45	0.46	0.41	0.41	0.38	0.38
Choline, mg/kg	1,700	1,801	1,700	1,700	1,700	1,700
Linoleic 18:2	1.26	1.16	1.60	1.27	1.72	1.50

Abbreviations: CP, crude protein; RCP, reduced protein; SCP, standard protein.

[1]Vitamin premix supplied the following per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 5,000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 mg; biotin, 200 mg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg.

[2]Trace mineral premix supplied the following per kilogram of diet: Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg.

where SD_p is the standard deviation of BWG, FI, or FCR within a treatment group; and $\bar{x}_p$ represents the mean values of the performance parameters.

Table 2. Analyzed amino acid values for the SCP and RCP finisher diets.

	SCP (mg/g DM)	RCP (mg/g DM)
Histidine	3.95	3.36
Serine	6.84	5.78
Arginine	8.74	8.29
Glycine	5.34	4.56
Aspartic acid	12.84	10.06
Glutamic acid	32.72	29.46
Threonine	6.02	5.58
Alanine	6.80	5.81
Proline	9.71	8.94
Lysine	8.60	8.21
Tyrosine	4.04	3.40
Methionine	3.37	3.74
Valine	7.04	6.81
Isoleucine	6.42	5.67
Leucine	12.26	10.41
Phenylalanine	7.54	6.37

Abbreviations: RCP, reduced protein; SCP, standard protein.

To determine flock BW uniformity %, the individual body weights of birds at d 34 were measured and the uniformity was calculated according to the following equation:

$$\text{Uniformity}\% = \left(1 - (SD_b/\bar{x}_b)\right) \times 100. \quad (2)$$

where SD_b is the standard deviation of BW of the birds for each treatment group; and $\bar{x}_b$ represents the BW mean across the treatment groups.

Statistical Analysis

The data was tested for their normality in distribution and the normally distributed data was analyzed according to a 2 × 3 factorial arrangement of treatments for the pen based measurements, and 2 × 2 × 2 factorial arrangement of treatments for the individual bird body weights, using the General Linear Model procedure of SPSS 24 package (IBM, Armonk, NY).The main effects included in the 2 × 3 factorial were rearing method (male single-sex, female single-sex, or equally mixed-sex) and CP level, and the main effects included in the

2 × 2 × 2 factorial were rearing method (single-sex or equally mixed-sex), sex, and CP level. Mean values of the treatments were compared within the confidence interval adjusted by Tukey test. All significant differences were determined at $P < 0.05$.

RESULTS

There was no difference in mortality between treatments. The effects of rearing method and dietary CP level on broiler performance in the period from d 0 to 10 are presented in Table 3. CP level significantly affected bird BWG ($P < 0.001$) and FCR ($P < 0.001$) regardless of the rearing method during the starter phase, where the RCP diet decreased BWG and increased FCR compared to the SCP diet. However, FI was not significantly affected by CP level ($P > 0.05$). Rearing method also showed a significant effect on BWG ($P < 0.001$) and FI ($P < 0.01$) regardless of CP level, where single-sex male pens had higher BWG and FI compared to both female only and mixed-sex pens. There was no significant difference between mixed-sex pens and female-only pens for BWG and FI ($P > 0.05$). Rearing method had no significant effect on FCR ($P > 0.05$). There was no significant interaction between rearing method and protein level during this phase ($P > 0.05$).

The effects of rearing method and dietary CP level on broiler performance in the period from d 11 to 24 are presented in Table 4. During the grower phase, CP level significantly affected FI and FCR ($P < 0.001$) regardless of rearing method, where the RCP diet reduced FI and increased FCR compared to the SCP diet. Rearing method also had a significant effect on FI ($P < 0.001$) and FCR ($P < 0.01$) regardless of CP level, where single-sex male pens had higher FI than other 2 groups and lower FCR than female-only birds. On the other hand, no difference of FI was observed between mixed-sex pens and female-only pens ($P > 0.05$), and no difference of FCR was observed between mix-sex birds with both male-only and female-only pens. There was a significant interaction between rearing method and protein level on BWG ($P < 0.05$) during this phase, where mixed pens showed significantly higher BWG than the female-only pens and lower BWG than the male-only pen when SCP was fed, but not when RCP was fed. However, BWG of male-only pens was significantly higher than female pens when birds were fed both SCP and RCP diets.

The effects of rearing method and dietary CP level on broiler performance in the period from d 25 to 35 are presented in Table 5. Crude protein level had a significant

Table 3. Performance of broiler chickens from d 0 to 10 in response to different rearing methods and dietary protein level.

Gender	CP level	BWG (g)	FI (g)	FCR
Male	SCP	255	293	1.151
	RCP	242	293	1.210
Female	SCP	241	280	1.160
	RCP	237	284	1.197
Mix	SCP	247	283	1.146
	RCP	234	283	1.211
Main effects				
Rearing method				
Female		239[b]	282[b]	1.178
Male		248[a]	293[a]	1.181
Mix		241[b]	283[b]	1.178
CP level				
RCP		238[b]	287	1.206[a]
SCP		248[a]	285	1.153[b]
P-value				
Rearing method	< 0.001	0.003	0.954	
CP level	< 0.001	0.632	< 0.001	
Rearing method × CP level	0.061	0.800	0.185	

Abbreviations: BWG, body weight gain per bird; CP, crude protein; FI, feed intake per bird; FCR, feed conversion ratio; RCP, reduced protein; SCP, standard protein.

[a,b]Means in a column not sharing the same superscripts are significantly different according to Tukey test ($P < 0.05$).

Table 4. Performance of broiler chickens from d 11 to 24 in response to different rearing methods and dietary protein level.

Gender	Protein level	BWG	FI	FCR
Male	SCP	1,138[a]	1,591	1.398
	RCP	999[c]	1,494	1.496
Female	SCP	994[c]	1,454	1.463
	RCP	925[d]	1,427	1.544
Mix	SCP	1,062[b]	1,509	1.420
	RCP	946[cd]	1,437	1.521
Main effects				
Rearing method				
Female		960	1,440[b]	1.504[a]
Male		1,069	1,542[a]	1.447[b]
Mix		1,004	1,473[b]	1.470[ab]
CP level				
RCP		957	1,452[b]	1.520[a]
SCP		1,065	1,518[a]	1.427[b]
P-value				
Rearing method	< 0.001	< 0.001	0.002	
CP level	< 0.001	< 0.001	< 0.001	
Rearing method × protein level	0.040	0.095	0.784	

Abbreviations: BWG, body weight gain per bird; CP, crude protein; FI, feed intake per bird; FCR, feed conversion ratio; RCP, reduced protein; SCP, standard protein.

[a,b,c,d]Means in a column not sharing the same superscripts are significantly different according to Tukey test ($P < 0.05$).

Table 5. Performance of broiler chickens from d 25 to 35 in response to different rearing methods and dietary protein level.

Gender	Protein level	BWG	FI	FCR
Male	SCP	1,215	2,125[a]	1.749
	RCP	1,155	2,025[ab]	1.756
Female	SCP	1,028	1,901[b]	1.848
	RCP	1,041	2,026[ab]	1.946
Mix	SCP	1,142	2,037[ab]	1.786
	RCP	1,074	1,970[ab]	1.834
Main effects				
Rearing method				
Female		1,035[c]	1,963[b]	1.897[a]
Male		1,185[a]	2,075[a]	1.753[b]
Mix		1,108[b]	2,003[ab]	1.81[b]
CP level				
RCP		1,090[b]	2,006	1.845[a]
SCP		1,128[a]	2,021	1.794[b]
P-value				
Rearing method	< 0.001	0.039	< 0.001	
Protein level	0.047	0.688	0.015	
Rearing method × protein level	0.162	0.026	0.195	

Abbreviations: BWG, body weight gain per bird; CP, crude protein; FI, feed intake per bird; FCR, feed conversion ratio; RCP, reduced protein; SCP, standard protein.

[a,b]Means in a column not sharing the same superscripts are significantly different according to Tukey test ($P < 0.05$).

Table 6. Performance of broiler chickens from d 0 to 35 in response to different rearing methods and dietary protein level.

Rearing method	CP level	BWG (g)	FI (g)	FCR
Male	SCP	2,608[a]	3,835	1.471
	RCP	2,397[b]	3,672	1.533
Female	SCP	2,263[c]	3,481	1.538
	RCP	2,204[c]	3,471	1.576
Mix	SCP	2,452[b]	3,687	1.504
	RCP	2,254[c]	3,502	1.553
Main effects				
Rearing method				
Female		2,234	3,476[c]	1.557[a]
Male		2,502	3,753[a]	1.502 [c]
Mix		2,353	3,594[b]	1.529[b]
CP level				
RCP		2,285	3,548[b]	1.554 [a]
SCP		2,441	3,667[a]	1.504[b]
P-value				
Rearing method	< 0.001	< 0.001	< 0.001	
CP level	< 0.001	0.001	< 0.001	
Rearing method × CP level	< 0.010	0.065	0.548	

Abbreviations: BWG, body weight gain per bird; CP, crude protein; FI, feed intake per bird; FCR, feed conversion ratio; RCP, reduced protein; SCP, standard protein.

[a,b,c]Means in a column not sharing the same superscripts are significantly different according to Tukey test ($P < 0.05$).

Table 8. Effect of sex, rearing method, and CP level on individual body weight of broilers at d 34.

CP level	Sex	BW
RCP	Female	2,101[d]
	Male	2,325[b]
SCP	Female	2,199[c]
	Male	2,535[a]
SEM		18.5
Source	*P*-value	
Sex	<0.001	
Rearing method	0.649	
CP level	<0.001	
Sex × Rearing method	0.255	
Sex × CP level	0.009	
Rearing method × CP level	0.130	
Sex × Rearing method × CP level	0.448	

Rearing method = male single-sex, female single-sex or equally mixed-sex.

Abbreviations: BW, body weight; RCP, reduced protein; SCP, standard protein.

[a,b,c,d]Means in a column not sharing the same superscripts are significantly different according to Tukey test ($P < 0.05$).

effect on BWG ($P < 0.05$) and FCR ($P < 0.05$) with the RCP diet resulting in lower BWG and higher FCR compared to the SCP diet. Rearing method had a significant effect on BWG ($P < 0.001$), and FCR ($P < 0.001$) as well. Single-sex male pens had significantly higher BWG than the single-sex female and mixed-sex pens. Single-sex female pens had a significantly higher FCR than the single-sex male and mixed-sex pens, whereas the FCR of the 2 latter rearing methods did not differ significantly. No interactions between the rearing method and CP level were observed for BWG and FCR. There was a significant interaction between rearing method and protein level on FI ($P < 0.05$) during this phase, where single-sex male pens showed higher FI than single-sex female pens only when the birds were fed SCP but not RCP diets. Mixed-sex pens did not show a difference from either single-sex male or female pens regardless of CP levels.

The effects of rearing method and dietary CP level on broiler performance during the overall period of the trial (d 0−35) are presented in Table 6. The RCP diet significantly affected bird performance by reducing FI ($P < 0.001$) and increasing FCR ($P < 0.001$) compared to the SCP diet. Rearing method also had a significant effect on FI and FCR ($P < 0.001$) with single-sex male pens having the highest FI (3,753 g) and lowest FCR (1.502) followed by the equally mixed-sex pens and lastly the single-sex female pens. There was a significant interaction between rearing method and protein level on BWG ($P < 0.01$). The RCP diet significantly reduced BWG in the single-sex male pens and mixed-sex pens ($P < 0.001$), but did not affect BWG of single-sex female pens compared to SCP diet. On the other hand, mixed-sex pens had higher BWG than the female birds only when fed with SCP diets but this was not observed when RCP diets were fed.

The effects of rearing method on broiler performance CV% based on the mean values per treatment in the periods from d 0 to 35 are presented in Table 7. The equally mixed-sex pens had the lowest CV% for BWG (2.61%), FI (2.41%) and FCR (1.69%) while the single-sex female pens had the highest CV% for BWG (3.07%), FI (3.18%), and FCR (2.05%). It is worthy to note that the CV% was calculated based on the mean pen BW of the treatments thus no replication was possible.

The effects of bird sex, rearing method and dietary CP level on individual d 34 birds BW with a 2 × 2 × 2 factorial design are presented in Table 8. Rearing method did not have a significant effect on d 34 body weight ($P > 0.05$) and no interactions occurred between the other two factors. However, there was a significant interaction between sex and CP level ($P < 0.01$). There was a significant difference between the mean d 34 BW of males and

Table 7. Coefficient of variation (%) of performance data of broiler chickens from d 0 to 35 in response to different rearing methods.

Rearing method	CP level	BWG CV (%)	Mean	FI CV (%)	Mean	FCR CV (%)	Mean
Male	SCP	2.656	2.91	2.621	5.71	2.255	1.72
Male	RCP	3.156		3.086		1.175	
Female	SCP	3.594	3.07	4.147	6.35	1.600	2.05
Female	RCP	2.544		2.203		2.491	
Mix	SCP	3.614	2.61	2.735	4.83	1.820	1.69
Mix	RCP	1.600		2.093		1.555	

Abbreviations: BWG, body weight gain per bird; CP, crude protein; FI, feed intake per bird; FCR, feed conversion ratio; RCP, reduced protein; SCP, standard protein.

Table 9. Uniformity (CV%) of male and female birds in response to different rearing methods and CP levels at d 34.

Gender	CP level	Rearing method	Bird uniformity (%)
Female	SCP	Single	90.51
		Mix	89.20
	RCP	Single	90.24
		Mix	91.71
Male	SCP	Single	91.55
		Mix	91.91
	RCP	Single	89.08
		Mix	90.47

females both within and between dietary crude protein levels. Although the RCP diet reduced the mean d 34 BW of both males and females, the extent of the reduction differed with the BW of males fed the RCP diet reducing by 209 g compared to those fed the SCP diet whereas the BW of females fed the RCP diet only reduced by 98 g.

The d 34 BW uniformities of male and female birds were examined according to rearing method and CP level (Table 9). The female birds reared as single-sex fed the RCP diet had a higher BW uniformity (90.24%) compared to the male birds reared as single-sex also fed the RCP diet (89.08%). The opposite occurred when the SCP diet was fed, where the single-sex male birds had a higher BW uniformity (91.55%) compared to the single-sex female birds (90.51%). The birds reared as mixed-sex had higher BW uniformities compared to those reared as single-sex with the exception being for females fed the SCP diet.

The d 35 relative meat and fat yield in male and female broilers fed diets of different protein levels is presented in Table 10. Dietary protein level had a significant effect on relative breast and drumstick weights with birds fed the RCP diet having significantly lower breast weights ($P < 0.001$) and higher drumstick weights ($P < 0.01$). There was a significant interaction between sex and protein level ($P < 0.05$) on relative fat pad weights where male birds had a lower fat pad weight compared to female birds, but this difference was only significant when the male birds were fed the SCP diet. There was no significant effect of sex or protein level on relative thigh weights.

Table 10. Relative meat and fat yield (%) of male and female broilers in response to different dietary protein levels on d 35.

Gender	Protein level	Breast	Thigh	Drumstick	Fat pad
Male	SCP	7.53	4.53	4.04	0.90[b]
	RCP	6.54	4.77	4.23	1.10[ab]
Female	SCP	7.60	4.66	4.02	1.21[a]
	RCP	6.67	4.66	4.25	1.17[a]
Main effect					
Sex					
Male		7.03	4.65	4.14	1.00
Female		7.14	4.66	4.14	1.19
Protein level					
SCP		7.56[a]	4.60	4.03[b]	1.06
RCP		6.61[b]	4.72	4.24 [a]	1.14
P-value					
Sex		0.634	0.861	0.987	0.002
Protein level		< 0.001	0.181	0.003	0.154
Sex × Protein level		0.906	0.171	0.678	0.042

Abbreviations: CP, crude protein; RCP, reduced protein; SCP, standard protein.

[ab]Means in a column not sharing the same superscripts are significantly different according to Tukey test ($P < 0.05$).

DISCUSSION

Overall the average growth performance of the birds fed the SCP diet in the present study exceeded 2018 Cobb-500 performance objectives for weight gain (2,263 g vs. 2,153 g for females and 2608 g vs. 2,392 g for males). The aim of this study was to determine the effect of rearing method (single-sex or equally mixed-sex) on the performance parameters and meat yield of broiler chickens using different dietary CP levels to detect variation between treatments. In addition, the effect of rearing method on broiler performance, CV % and bird uniformity was investigated.

It is well known that sex has a significant effect on performance with males having higher BWG, FI and better FCR compared to females (Shafey et al., 2013; Benyi et al., 2015; Da Costa et al., 2017; Madilindi et al., 2018). The results from our experiment confirmed this, as male birds reared as single-sex had the highest average BWG and FI followed by the birds reared in equally mixed-sex pens and lastly the female birds reared as single-sex. Sex also had a significant effect on FCR, as expected, especially during the later feeding phases where male birds reared as single-sex had the lowest FCR, followed by the birds reared in equally mixed-sex pens and lastly the female birds reared as single-sex. This result is supported by Benyi et al. (2015) while some other studies reported an insignificant effect of sex on FCR (Siaga et al., 2017; Madilindi et al., 2018). Such discrepancies may be due to the diets fed, breed or flock variations, rearing system, management, and possibly the extent of variation between the treatments resulting in a lower test power in some studies, thus no differences were detected. It may be justifiable to conclude based on our data that male broilers are more efficient in feed conversion compared to female broilers at least during the later stages of growth.

Interestingly our results did not show any significant effect of rearing method on d 34 BW. However, Da Costa et al. (2017) reported that males reared as mixed-sex had significantly higher growth rates compared to the males reared in single-sex pens. The opposite was observed in the study for the females where they performed better reared as single-sex compared to mixed-sex. Some studies have reported nonsignificant differences in BW of each sex (Smith et al., 1954; Lamoreux and Proudfoot, 1969), but it was observed that numerically males were heavier and females lighter when they were reared in the mix-sex pens compared to their single-sex counterparts. Although numerically different, there was no statistical difference between rearing methods in these experiments as well as in the present study. It was, therefore, apparent that females did not benefit from

single-sex rearing and males from being reared in mixed-sex pens. Da Costa et al. (2017) suggested that when females are reared as mixed-sex, the competition for feeder space was higher which could have resulted in lower feed intake, and, consequently, resulted in females that were reared alongside males being lighter than if they were reared separately. Their study was performed using birds of an older genetic line where growth rates were not as fast resulting in birds that had lighter final BW. It was, therefore, observed by Da Costa et al. (2017) that the birds were a lot more active and more inclined to interact with each other. However, it was observed in our study that as the birds grew and became heavier the less they moved around the pen meaning that even in the mixed-sex pens there would have been plenty of opportunities for all the birds to reach the feeders and males were not dominant over the females. It is also important to note that according to the present guidelines for better animal welfare, the birds are reared in much larger spaces with more sufficient feeder space compared to the settings in the past. Therefore, it is now unlikely that the competition for feed between birds is an issue and the females no longer face being pushed away from the feeders by the males.

The results from this study showed that females had lower CP requirements than males. This is shown by the effect of dietary CP level on the individual BW of the birds at d 34. The extent of the reduction in the mean BW of males was much greater than that of the females when the RCP diet was fed. Hernández et al. (2012) reported that a 1.5% reduction in dietary CP fed to Ross-308 broilers adversely affected the performance of the male birds, but not the female birds, whose CP could be reduced to 3% with no adverse effects. In addition, our study showed that there was no significant effect of CP level on fat-pad weight in females in fact the fat-pad weight actually decreased numerically when they were fed the RCP diet. However in males, the fat-pad weight significantly increased when they were fed the RCP diet which is an indicator of a higher energy intake compared to CP intake. When the effect of CP level on flock uniformity was examined, females fed the RCP diet had a higher BW uniformity compared to males fed the RCP diet reared as either single or mixed-sex. The opposite occurred when the SCP diet was fed where males had a higher BW uniformity compared to females. Additionally, the CV % for BWG and FI for the overall period of the experiment was lower, when females were fed the RCP diet compared to the SCP diet, while the opposite was observed for the males where the lower CV% for BWG and FI was achieved when they were fed the SCP diet. These results support the conclusion that the CP requirements for male and female birds are different and the CP requirements given by the breed companies may meet the requirement of males but not the females, due to the fact that the majority of nutritional studies in the past have been based on male broilers. Good flock uniformity is an important goal of both researchers and producers as it allows for easier optimization of feed and feeding programmes as well as reduced variability in the final product which makes bird processing easier. This is where separate-sex rearing may indeed be of benefit as it will allow for precision feeding which will help to more accurately meet the requirements of the different sexes, thereby improving growth rate, feed efficiency, and BW uniformity (Gous, 2017).

The next important finding from our study is that rearing birds as equally mixed sex resulted in a lower performance CV% and higher BW uniformity compared to rearing birds as single-sex. This result is not in agreement with other literature (Lang et al., 1960; Gehle et al., 1974; Da Costa et al., 2017) who reported that single-sex pens have higher BW uniformities compared the pens that had equal numbers of male and female birds. Certainly there are a number of other factors that could have an effect on the results such as the breed used and diets as well as other random variations. It may be worthwhile investigating this further in future, but for now our results show promise for researchers that are unable to source single-sex birds for use in experiments to minimize variation. It is possible to use mixed-sex birds with equal number of males and females to provide the same between-pen variation as single-sex bird experiments.

Lastly, from our relative meat yield results we can conclude that when birds are fed diets low in CP, they will prioritize the growth of more important body parts such as the legs. It was observed that CP level had a significant effect on relative breast and drumstick weights where birds fed the RCP diet had lower breast but higher drumstick weights. Van Harn et al. (2019) reported similar results where a reduction in CP resulted in lower relative breast but higher relative leg weights in Ross-308 broilers. A possible reason for this could be due to the different responses of different fibre types to low CP diets. (Li et al., 2016) found that in growing pigs fed a low CP diet, there was a difference in the expression of certain muscle development regulatory genes when 2 different muscles were compared, meaning the low CP diet would affect the growth of these muscles differently. It could also be that protein use is prioritized to the tissue where the animal's survival is dependent on, for example, legs, essential for the animal's ability to walk, and therefore, have access to feed and water. Therefore, when the body is faced with a limiting nutrient such as is the case when a diet low in CP is fed, then priority will be given to the growth of the legs.

Overall, in the current study, male broilers outperformed female broilers and our results have shown that females have lower CP requirements compared to males and feeding males and females separately to more accurately meet their nutrient requirements can help improve performance and BW uniformity. There is also the potential that rearing broilers as equally mixed-sex will have a lower between-pen variation compared to broilers reared as single-sex, this is of importance especially since it is becoming increasingly difficult to source single-sex birds for research purposes. Lastly, we made the observation that when low CP diets are fed, birds prioritize the growth of more important body parts such

as the legs over less important ones such as breast tissue. More in-depth research is needed to determine the exact mechanisms underlying such prioritizing use of protein for legs when lower protein diets are fed to broilers.

DISCLOSURES

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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