Estimation of genetic distances and heterosis in three ostrich (*Struthio camelus*) breeds for the improvement of productivity

By

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Dedication

I dedicate this thesis to Jesus Christ, may You be glorified through this work, and to all the youth in Franschhoek as a inspiration to rise from their circumstances and to know that nothing is impossible with God.

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

A study was conducted to characterize the three ostrich breeds available as genetic resource in South Africa, namely the South African Black (SAB), Zimbabwean Blue (ZB) and the Kenyan Redneck (KR), and their respective crosses. Growth, slaughter traits and reproduction of these ostriches were recorded at Oudtshoorn Research Farm in the Western Cape of South Africa. Individual non-linear regressions (Gompertz) were fitted to the data of 390 purebred and 41 crossbred ostriches, using the SAS NLIN function. Heterosis was estimated for each parameter of the Gompertz model. The estimated adult weight (A-parameter) of the ZB (147 kg) and the KR breeds (148 kg) were higher than that of the SAB breed (129 kg). The overall growth rate (B-parameter) of the ZB breed (0.0075) and the SAB breed (0.0080) was lower than that of the KR (0.0150). The age at maximum weight gain (C-parameter) was higher for the ZB breed (226 days) compared to the SAB (198 days) and the KR (194 days). Heterosis for the A-parameter was estimated at -6.2% and at -12% for the C-parameter. The slaughter traits studied were slaughter weight (SLW), carcass weight (CW), dressing percentage (DP), fan fillet weight (FFW), pH0, pH24, drip loss % (DL%), cooking loss % (CL%), tenderness and meat colour traits. Differences were observed between the means for SLW of the SAB (86.5 kg) and ZB (93.9 kg). Mean DP of the KR breed (52.5%) was increased relative to the low DP of their SAB contemporaries (48.8%). The sire lines (ZB and KR) and crosses were heavier than the SAB (dam line), whereas the crosses resembled the dam line for meat quality traits. Means for pH_{24} also differed, with higher values for the sire lines (ZB – 6.36; KR – 6.4) relative to the SAB (5.85). The instrumental b* colour value also differed between the SAB (9.4) and KR (6.9). Records used for assessing the reproduction and body measurements of purebred and crossbred dams were 428 in total. Traits analyzed were, total egg production (TEP), the number of fertile eggs, number dead in shell chicks, hatchability and chick production (CP), the time to lay, live weight, front chest circumferences as well as tail circumference. The ZB and KR were heavier in live weight and of larger body measurements than the SAB, whereas the SAB exhibited superior reproduction performance in comparison with the ZB and KR breeds. Derived heterosis estimates amounted to 2.2% for tail circumference, 12% for TEP, 12% for hatchability and 19% for CP. Genetic variation between and within the breeds were determined utilizing 19 microsatellite markers. Significant molecular genetic differences were observed between the three breeds. The SAB and ZB (Fst = 0.10 and Nei = 0.49) were genetically most similar, whereas the genetic distance between the KR and ZB breeds were furthest (Fst = 0.13 and Nei = 0.61). The SAB breed exhibited the highest heterozygosity within its population and the ZB the lowest heterozygosity. These results contribute to a better understanding of the utilization of the distinct ostrich breeds for commercial production.

Opsomming

Die doel van hierdie studie was om die verskille tussen drie volstruisrasse wat tans in Suid Afrika mee geboer word te karakteriseer, naamlik die “South African Black” (SAB), “Zimbabwean Blue” (ZB) en die “Kenyan Redneck” (KR) en hulle onderskeie kruisse. Rekords van die groei-, slag- en reproduksie eienskappe van die volstruise was by Oudtshoorn Navorsingsplaas in die Wes-Kaap aangeteken. Individuele nie-lineêre regressies (Gompertz) is op die data van 390 suiwerras en 42 kruisgeteelde volstruise gepas, met die gebruik van die “NLIN” prosedure van SAS, (2006). Heterose is beraam vir elke parameter van die Gompertz model. Die beraamde volwasse gewig (A-parameter) van die ZB (147 kg) en die KR ras (148 kg) was hoër as die van die SAB ras (129 kg). Die totale groeitempo (B-parameter) van die ZB ras (0.0075) en die SAB ras (0.0080) was laer as die van die KR (0.0150). Die ouderdom by maksimum groei (C-parameter) was hoër vir die ZB ras (226 dae) in vergelyking met die SAB (198 dae) en die KR (194 dae). Heterose vir die A-parameter was beraam teen -6.2% en teen -12% vir die C-parameter. Die slageienskappe wat ondersoek was, was slagmassa (SLW), karkasmassa (CW), uitslag persentasie (DP), “fan fillet” massa (FFW), pH0, pH24, drupverlies % (DL%), kookverlies % (CL%), sagtheid en kleureienskappe. Beduidendende verskille is waargeneem tussen die gemiddeldes vir SLW vir die SAB (86.5 kg) en ZB (93.9 kg). Gemiddelde DP van die KR ras (52.5%) was beter as die van die SAB ras (48.8%). Die mannetjielyne (ZB en KR) en die kruisse was swaarder as die SAB (wyfielyn), en die kruise was vergelykbaar met die wyfielyn vir vleiskwaliteit eienskappe. Gemiddeldes vir die pH_{24} het verskil, met hoër waardes vir die vaar lyne (ZB – 6.36; KR – 6.4) relatief tot die SAB (5.85). Die instrumentale b* kleurwaarde het ook verskil tussen die SAB (9.4) en KR (6.9). ‘n Totaal van 428 rekords is gebruik om reproduksie en liggaamsmetings van die suiwer en kruisteelwyfies te ondersoek. Reproduksie eienskappe ge-analiseer was: die aantal broeisels, totale eierproduksie (TEP), die aantal vrugbare eiers, die aantal kuikens dood in dop, uitbroeibaarheid, kuiken produksie (CP), tyd tot produksie van die eerste eier, volwasse gewig, voorbors omtrek, sowel as, kruisomtrek. Die ZB en KR rasse was swaarder as die SAB, en het groter liggaamsmetings gehad. Die SAB het beter reproduksie in vergelyking met die ZB- en KR rasse gehad. Heterose beramings was 2.2% vir kruisomtrek, 12% vir TEP, 12% vir uitbroeibaarheid en 19% vir CP. Genetiese variasie tussen en binne die rasse was vasgestel deur die gebruik van 19 mikrosatelliete merkers. Beduidende genetiese verskille op ‘n molekulêre vlak was waargeneem tussen die drie rasse. Die SAB en ZB (Fst = 0.10 en Nei = 0.49) was geneties meer gelyk terwyl die KR en ZB genetiese verder verwyder is (Fst = 0.13 en Nei = 0.61). Die SAB ras het die hoogste heterosigositeit binne populasie getoon, en die ZB die laagste. Hierdie resultate dra by tot ‘n beter begrip van die gebruik van die drie rasse in kommersïele produksie.

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Chapter 1

Introduction

Background

Ostrich farming originated in South Africa during the 1800's and was originally renowned for quality feather production (Deurden, 1913). The Northern African Ostrich and the Southern Ostrich were crossed to develop a breed specializing in feather production, now known as the South African Black (SAB- *Struthio camelus domesticus*). However the importance of feathers has decreased since World War II due to the changes in the fashion industry. Currently feather production only accounts for 5%, leather 33% and meat 62% of the ostrich production (personal communication 2010, South African Ostrich Business Chamber, email: akruger@saobc.co.za).

The goal of most commercial poultry farmers is to attain high egg production in layer breeds, while fast growth rate and good feed conversion ratio are sought after in broilers to ensure a higher profit with less input costs. In contrast, ostrich production centers around the hatching of high numbers of high quality day-old chicks, with a efficient survival rate and favourable growth characteristics to slaughter (Cloete *et al.*, 2002, 2008b). However in South Africa, there is a lack of breeding goals and structured breeding plans in ostrich breeding (Petite & Davis, 1999). According to Cloete *et al.* (2008b), there are no formal animal recording and genetic improvement scheme in place for South African ostriches. It can therefore be assumed that slow progress is made in the genetic improvement in ostriches. The direct consequence of this situation is that no measurable genetic gains are achieved in ostrich breeding flocks used for commercial production. It is known that ostriches exhibit sufficient additive genetic variation in the most important production traits (Cloete *et al.,* 2008a), while genetic change in reproduction of ostriches has been demonstrated in an experimental flock (Cloete *et al.,* 2008a). Seen against the difficulty of realizing sustainable additive gains in the commercial industry, the possible exploitation of breed differences and hybrid vigour in commercial breeding stock needs to be considered. It is therefore necessary to evaluate the different ostrich breeds currently farmed with in South Africa for their relative performance, the genetic distances between them, as well as for the combinability between breeds.

The three major ostrich breeds available for commercial production in South Africa are the South African Black (SAB), Zimbabwean Blue (ZB) and the Kenyan Redneck (KR). At present it is known that the SAB breed are capable of a superior reproductive performance relative to the ZB breed (Cloete *et al.*, 2008c) while the KR and ZB are thought to be superior for growth traits (Jarvis, 1998). However, definite breed characteristics, indicative of genetic differentiation, have not been quantified for each breed in terms of reproduction, growth and carcass traits. Crossing of these breeds are therefore occurring randomly without any appropriate breeding goals (Petite & Davis, 1999).

Justification

Crossbreeding is known to improve lowly heritable fitness traits, such as reproduction and survivability (Pirchner, 1969). The South African ostrich industry is characterized by poor egg and chick production, impaired hatchability and survivability of chicks (Cloete *et al.*, 2001, 2008b). Its therefore seems feasible to implement crossbreeding systems in the commercial ostrich industry in an attempt to alleviate the abovementioned problems.

Growth traits are also known to benefit from hybrid vigour in livestock species. Most of the studies conducted on growth traits and growth curves have been on the SAB breed. Some studies have compared the SAB and ZB breeds (Du Preez *et al.,* 1992; Sabbioni *et al.,* 1999), but no studies have compared the growth traits and growth parameters of the three ostrich breeds, SAB, ZB, KR as well as crosses of the ZB breed with the SAB. In the abovementioned study by Du Preez *et al.* (1992), the rate of maturing differed significantly between the SAB and ZB breed however their mature weight did not differ significantly from each other. The only results reporting a comparison between the three breeds for mature live weight, which could serve as a proxy for growth was those of Jarvis (1998) who reported the SAB (115 kg) to have the smallest mature live weight, compared against the ZB (125 kg) and KR (135 kg) breeds. Studies have been done to exploit direct heterosis for growth traits in ostriches by crossbreeding the SAB and ZB breeds. Heterosis estimates of 6.7% were derived for live weight at 14 months (used as an indication of slaughter weight) and for chick survival until 30 days of age (Engelbrecht *et al.*, 2008).

Crossbreeding is also known to improve slaughter traits. Quantitative and qualitative slaughter traits have been investigated for the SAB, ZB and the ZB male crossed to SAB females. It was shown that the ZB had a heavier slaughter weight than the SAB and the instrumental tenderness and sensory traits did not differ between these breeds (Brand, 2006; Hoffman *et al.*, 2008). However this information involved inadequate numbers (only two animals represented the ZB breed), while the KR breed has not been included. Further studies are thus necessary to confirm or refute these earlier preliminary studies.

Reproduction traits are mainly influenced by the maternal genetic component. Crossbred dams are also known to perform better than the average of purebred dams used to create the crossbred breed in other livestock species. The reproductive performance of the three South African ostrich breeds, the SAB, ZB and KR has not been meticulously described. Studies have been conducted on the reproductive performance of the SAB females compared to that of the ZB females' (Brand, 2006; Cloete *et al.,* 2008c). The SAB exhibited superior reproduction performance in both studies. However, the latter authors conceded that the ZB resource in these studies has been poorly quantified in terms of ancestry and female age. They recommended that further studies needs to be conducted using a better quantified resource of ZB birds. Moreover, no studies have been conducted on the performance of crossbred females and the effect of maternal heterosis on ostrich reproduction traits.

It is known that the probability of attaining high levels of heterosis in crossbred progeny is enhanced with greater genetic distances among breeds participating in a structured crossbreeding programme (Pirchner, 1969). Genetic distances between the South African ostrich breeds have not yet been studied in South

Africa. However, molecular methods have been used to determine genetic variation among ostrich subspecies in other countries. Such studies include those of Kawka *et al.* (2007), reporting that the KR and ZB were genetically more closely related, whereas the KR was further separated from the SAB than the ZB. However, the population used in the latter study was based in Poland and only five microsatellite markers were used. Further investigation is therefore needed using more microsatellite markers to determine the genetic variation between and within the South African populations of the SAB, ZB and KR genetic resources. It is unknown whether these results will be similar to those of Kawka *et al*. (2007) for the local commercial ostrich population. If substantial genetic distances are found between these breeds, it will provide theoretical backing for the development of crossbreeding systems for usage in the commercial ostrich industry.

Therefore the aim of this study is to compare, growth, carcass and reproduction traits of the three purebred breeds and mostly crosses involving the ZB and SAB. Maternal heterosis will be estimated for reproduction traits where appropriate. Direct heterosis will also be estimated for growth and carcass traits. The genetic variation between and within the breeds will also be assessed, based on genetic analysis of microsatellite markers. Thus the specific objectives of the study are:

- The estimation of breed effects (as indicative of genetic distances) as well as direct heterosis for the Gompertz growth parameters.

- The estimation of breed effects (as indicative of genetic distances) as well as direct heterosis and its effect on the following slaughter traits: slaughter weight, carcass weight, and fan fillet weight; dressing percentage, pH, cooking loss percentage, drip loss and instrumental colour.

- The estimation of breed effects (as indicative of genetic distances) as well as maternal heterosis for reproduction traits (total egg production, hatchability, number of dead in shell chicks, number of fertile eggs, chick production, time to lay).

- The determination of the genetic distances between three different ostrich breeds and the genetic variation within the three ostrich breeds, viz. the SAB, KR and the ZB, using microsatellites as markers.

Outcomes from these studies will be used as a starting point for a crossbreeding strategy for commercial ostrich production, based on scientific principles. It is foreseen that these principles will be expanded as time continues to act as a guideline for the optimal utilization of the ostrich genetic resource.

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Chapter 2

Literature Review

The ostrich

Ostriches were first domesticated in 1867 for the purpose of feather production (Douglass, 1881). The North African ostrich are known to yield a better feather quality than the South African birds and were imported in 1869 from Barbary (the middle and Western coastal regions of Northern Africa - Morocco, Algeria, Libya, Mauritania and the Western Sahara) through the Port Elizabeth harbour (Douglass, 1881). Crossing of the North African and the Southern African bird occurred to improve feather production and quality (Deurden, 1913). This hybrid breed is currently known as the South African Black (SAB).

Ostriches are considered as the world's largest living birds and grow up to 2.1 meter to 2.8 meter in height (Shanawany, 1995; Deeming, 1999). It has a two meter wingspan. Ostriches reach a mature weight of 100 to 160 kg depending on the subspecies.

Naturally ostriches are desert animals and can withstand hot temperatures up to 56°C. It can also adapt to other widely divergent climatic conditions (Shanawany, 1995). In South Africa they are mostly farmed in the Klein Karoo area which is a semi-arid area situated in the Western Cape Province, and in the South-western Cape. The area has a Mediterranean climate, with temperature of 24°C to 30°C during summer season and an annual rainfall of 300 to 400 mm per annum. They are gregarious animals and form groups of mixed gender and age during non-breeding season (Deeming & Bubier, 1999).

Taxonomy of the ostrich

The ostrich is classified as a ratite, paleognathic bird (Deeming, 1999). It belongs to the family Struthionidae and is classified as *Struthio camelus*. However different subspecies, breeds or breeds have been recognized in the wild (Jarvis, 1998). Five subspecies have been identified whereof one has become extinct in 1941. This subspecies was known as *S. c. syriacus*, and were found in the Middle East (Douglass, 1881, Jarvis, 1998; Deeming 1999). The other four subspecies are *S. c. australis* from southern Africa, *S. c. camelus* from northwestern Africa, *S. c. molybdophanes* from northern Africa, and *S. c. massaicus* from eastern Africa (Jarvis, 1998). Various breeds have been identified within those subspecies. For example the Zimbabwean, Namibian, Kalahari and West Coast ostrich breeds are all classified under the subspecies *S. c. australis* (Jarvis, 1998). As stated previously, the composite SAB breed is also identified as a separate subspecies, commonly referred to as *S. c. var. domesticus*. Different breeds have also been identified within the domesticated SAB breed as selected by breeders from different farms in South Africa; these breeds were referred to as the commercial line and the feather line (Bunter, 2002). However, no significant differences were observed between the abovementioned breeds in terms of reproduction traits (Bunter, 2002). Another breed, known as the Israeli Black has also been created, by crossing *S. c. domesticus* with *S. c. massaicus* and *S. c. molybdophanes* (Jarvis, 1996). These birds are thought to have better reproduction than the South African Blacks (Jarvis, 1996), although no references providing proof of this allegation could be sourced.

According to the latter author, this advantage may be due to the development of inbreeding depression in the farmed SAB population.

The largest and tallest subspecies are considered to be the east African ostrich (*S. c. massaicus*), weighing 135 kg, which is widely distributed on the African continent (Jarvis, 1998). This subspecies are also referred to as the Kenyan Redneck (KR), because of their prominent red neck color. The Zimbabwean blue (ZB - 125 kg) is categorized under the *S. c. australis* subspecies and is larger than the other ostrich breeds also classified as *S. c. australis* and abiding in the Southern and Western parts of Africa. The Namibian ostrich is for example also classified as *S. c. australis* but has smaller in live weight (100 kg) than the ZB. The Somalian Blue (*S. c. molybdophanes*) is also regarded as a breed with a lower live weight, at 105 kg. The SAB breed (*S. c. domesticus*) (115 kg) is a composite breed resulting from the cross between the North African and South African ostrich (Deurden, 1913), and was originally was known as the Feather Black.

Of the ostrich breeds listed above, only three are commonly used in commercial production in South Africa, and also in the rest of the world (Petite & Davis, 1999). These include the SAB, ZB and KR, which are described below (Table 2.1). In commercial production, these breeds are often used in haphazard combinations, often under colony mating management regimes with no performance recording (Petite & Davis, 1999). Although substantial size differences were reported between breeds in the study by Jarvis (1998), very few published studies are available where these breeds were compared in designed experiments. A number of studies were conducted where the SAB and ZB breeds were compared (Brand, 2006; Cloete *et al.*, 2008b; Engelbrecht *et al.*, 2008; Hoffman *et al.*, 2008), but no studies involving the KR could be sourced. Knowledge of breed or breed differences is cardinal for defining the position of specific breed in the commercial production process, as well as genetic distances among breeds. The existing knowledge on the available ostrich genetic resource thus needs to be broadened to allow sound decisions on a scientific and economic base.

Table 2.1 Phenotypical differences between the three ostrich breeds commonly used in the South African commercial ostrich industry (as adapted from Jarvis, 1998).

Feature	SAB	Kenyan Red	Zimbabwean Blue
Crown (Bald)	Yes/No	No	No
Neck Skin (color male)	Grey	Pink	Grey
Neck collar (White)	Variable	None	Thin
Neck bare	No	Yes	No
Eye colour	Brown	Brown	Brown
Body Skin (male)	Grey/Blue	Pink	Grey/Blue
Body skin (female)	Brown	Brown	Brown
Leg scutes (males)	Red	Red	Red
Tail feathers (male)	Rusty or white	Pale Brown	Rusty
Tail feather (female)	Brown	Brown	Brown
Adult weight	115 kg	135 kg	125 kg

Breeding systems in the ostrich industry

The onset of the breeding season is synchronized photoperiodically and coincides with increasing daylight in birds (Hafez & Hafez, 2000). This mechanism is also applicable to ostriches (Deeming & Bubier, 1999, Lambrechts, 2004). The South African breeding season starts during May/June to December/January/February. Wild ostriches reach sexual maturity at three to five years, while farmed ostriches reach sexual maturity at two to three years (Shanawany, 1995; Petite & Davis, 1999). Females reach sexual maturity before males (Shanawany, 1995; Deeming & Bubier 1999). The plumage of the ostrich sexes is dimorphic in adults, with males having vividly black and white plumage. In contrast, the plumage of females is duller, with a grayish-brown colour (Shanawany, 1995).

In their natural environment, ostriches are group breeders and utilize a communal nesting system (Deeming, 1999, Kennou Sebei *et al.,* 2009). Commercial farming systems therefore need to be adapted to consider this peculiarity of the species. South African farmers use a combination of pair or trio breeding and colony breeding and in other countries pair or trio breeding systems are mainly used (Petite & Davis, 1999). Colony breeding is the keeping of males and females together in a group for breeding purposes. The ratio between sexes commonly used by commercial South African farmers is five to six males for every ten females, and these breeding flocks can range in size from 50 to 200 birds (Deeming & Bubier, 1999). The gender ratio for breeding birds in colonies is normally six males to ten females (Lambrechts *et al.,* 2004). Literature reported that fertility and hatchability of ostriches are not negatively influence with a ratio of one male to two or three females (Lambrechts *et al.,* 2004).

The problem with colony breeding is that it is impossible to identify the parents of the chicks, as well as the reproductive capacity of individual females. Because of this constraint, there is a lack or mostly a total absence of pedigree recording on South African farms. The disadvantage of pair breeding is that the genetic

effects of males and females are confounded. Popular opinion in farmer communities also held the thought that the same combinations must be paired off repeatedly under commercial farming conditions (Cloete *et al.,* 1998). Pairs are often kept in the same breeding paddock for almost 20 years, also resulted in confounding random animal effects with random breeding paddock effects, while also extending the generation interval (Cloete *et al.,* 1998). This can be overcome by rotating the sires and dams between pairs and breeding paddocks from breeding season to breeding season.

The South African ostrich industry

Commercial ostrich farming first began in South Africa in 1864 (Smit, 1963). The ostrich industry predominantly concentrated on feather production until 1914 (Van der Vyver, 1992). Feather production declined at the onset of World War I, because of a disruption to exporting, inadequate marketing as well as a revolution in fashion of that day and age. By 1930 the commercial South African ostrich population declined from 770000 head to 23000 head. After the demise of the feather trade, the emphasis of ostrich farming shifted towards meat and leather production, which contributed 90% or more of overall commercial production in recent years. Leather contributed 70%, meat 25% and feathers 5% in the mid 1990's (Cloete *et al.*, 1998). Subsequently, this scenario has changed to meat and leather contributing 45% each to the income from a slaughter bird to farmers (Cloete *et al.*, 2002). Currently the overall contribution of ostrich products to the ostrich farmers are 3% for feathers, 37% leather and 60% meat production (personal communication 2010, South African Ostrich Business Chamber, email: akruger@saobc.co.za). This increased demand is mainly due to the fact that ostrich meat is considered healthier than other red meat because of its low intramuscular fat content (Sales, 1996).

A one channel co-operative marketing system was established by the Klein Karoo Agricultural Co-operative (KKAC) during 1959 (Van der Vyver, 1992). The KKAC owned the first abattoir that was established in 1960, and which is located in Oudtshoorn. The one-channel marketing system was abolished in 1993, and this led to an increase of ostrich meat exports as well as an increase in ostrich prices (going at R1440/bird) until 1996 (Van Rooyen *et al.*, 1998). During 1997, there was a reduction in ostrich product prices. This might have been due to an oversupply in leather products and the marketing not being able to keep up with the leather supply. In 2010 the average price for per breeder bird increased to R3500 (personal communication 2010, South African Ostrich Business Chamber, email: akruger@saobc.co.za).

At present (December 2010), there are 370 local ostrich farms registered for export to the European Union. These farms produced 263000 slaughter birds in 2009/2010 (personal communication, South African Ostrich Business Chamber, email: akruger@saobc.co.za). There are also approximately 700 specialist chick rearing farms to assist with the production process. There are ten abattoirs approved for export of ostrich meat products, and 15 tanneries finishing ostrich leather in South Africa. The ostrich industry provides employment for almost 20000 workers. Van Rooyen *et al.* (1998) cited a study done by Eckert & Liebenburg (1997) that assessed the impact of the different economic sectors in the Western Cape. From the industries studied, the ostrich industry were 4th for earning foreign exchange, 17th for added value, 18th for employment creation and 39th for income redistribution (Van Rooyen *et al.*, 1998). Overall the ostrich industry was

concluded to be in the 17th place of all the other industries adding to the economy of Western Cape. In recent years the ostrich industry also contributed ~R2.1 billion per annum to the South African economy (personal communication, South African Ostrich Business Chamber, email: akruger@saobc.co.za). It can therefore be concluded that the ostrich industry plays an important role in the economy of South Africa, and particularly in the regional economy of the Western Cape (Van Rooyen *et al.*, 1998).

Problems in the ostrich industry

Despite the importance of ostrich production to the economy of South Africa, there are a certain constraints impeding optimal production. Currently there are no structured breeding strategies with proper breeding goals (Cloete *et al.,* 1998; Petite & Davis, 1999; Cloete *et al.,* 2008a). Since meat and leather products contribute the most income to producers, breeding goals to improve these traits must be set. Cloete *et al.* (2002; 2008a) suggested focusing on the number of good quality chicks surviving to slaughter, their growth and their feed efficiency. These are considered as important traits to produce ostrich products in a sustainable and cost-effective way. The quantity of eggs and chicks produced, as well as day-old chick weight, need to be recorded if sustained genetic improvement of the national or international breeding flock is envisaged. Another way to improve the structure of the commercial industry is to create specialist lines that are selected for reproduction, as well as for growth and meat traits. Under this scenario the dam lines can be selected for reproduction while sire lines can be selected for growth, meat and skin traits (Cloete *et al.*, 1998; Petite & Davis, 1999; Cloete *et al.*, 2002). Essential traits to select for in the female line are: age at sexual maturity and chick production. In contrast, objectives in the sire line may include traits like bodyweight as a proxy for growth, feed efficiency, lack of aggressiveness (for easier handling by stockman), and fertility.

Genetic parameters i.e. heritabilities, repeatabilities and genetic correlations are important measures that are used by animal breeders to achieve genetic gain within a population (Gowe & Fairfull, 1995). Information has been obtained on the area of parameter estimates for production traits in ostriches, from studies done by van Schalkwyk *et al.* (1996) and Cloete *et al.* (1998). These genetic parameters have been extensively reviewed by Cloete *et al.* (2008a). However, comparing this information with scientific work done in other livestock species indicates there is still a lack of information on genetic parameters for key production traits, and in some cases of environmental influences on production in ostriches (Cloete *et al.*, 1998; Bunter, 2002; Cloete *et al.,* 2002, 2008a). It was also concluded that the bulk of genetic parameters available to guide ostrich breeding was derived from the same pair-breeding ostrich flock at the Oudtshoorn Research Farm (Cloete *et al.,* 2002, 2008a) and can thus not be used as an indication of the national population. The addition of parameter estimates from future studies will add to the robustness of genetic parameters reported for the species.

Ostriches perform less than optimally where fitness and reproduction traits are of concern in comparison to other livestock species for e.g. broiler breeders females that attains a total egg production (at 58 weeks of age) of 67 for broiler selected for high growth and 102 eggs for broilers that were selected for low growth (Renema *et al.,* 2006). Suboptimal performance in these traits is reflected in a high chick mortality rate (Cloete *et al.,* 2001), high levels of embryonic deaths, resulting in impaired hatchability of eggs (Brand *et al.,*

2007), and a relatively low and variable egg production rate (Cloete *et al.,* 2002; 2008b). The artificial incubation of ostrich eggs remains challenging, and chick output is constrained by a low hatchability, a high number of infertile eggs and shell deformities (Deeming *et al.*, 1993; Smit *et al.*, 1995; Van Rooyen *et al.*, 1998). Variable coefficients of variation (CV's) have been reported for fertility of ostriches ranging from 30%-80% and 11%-80% for hatchability (Deeming & Ar 1999, Bunter & Graser, 2000). Therefore it can be seen that there is a high variation in reproduction traits. This variation in egg production, hatchability and chick weight at specific ages is influenced by the genetic composition of the breeding pairs (Cloete *et al.*, 1998).

Reproduction is the very basis of livestock production in all domesticated mammalian and avian species. In the past, reproduction and fitness traits were largely neglected in commercial livestock breeding enterprises; and the focus was mainly on growth and other qualitative and quantitative production traits. Reproduction is known to be negatively correlated with growth traits in some species for e.g. in broiler breeder birds where they have been selected for increase body weight which have a negative effect on their fertility (Decuypere *et al.,* 2010). Fitness traits are known to decline with the selection for other production traits (Goddard, 2009). Fitness traits can be defined as traits that measure survival and reproduction rate in animals (Goddard, 2009). The latter can be observed in broiler breeders where selection for an increased growth rate resulted in a reduction in reproductive efficiency (Hocking *et al.,* 1989). Furthermore, inbreeding depression has previously been linked with low fitness and a reduced survival of livestock (Falconer & Mackay, 1996). Lowly heritable fitness traits often benefit from non-additive gene action leading to heterosis (Pirchner, 1969). Therefore it can be argued that crossbreeding can alleviate the effect of inbreeding depression, and result in increased reproduction and fitness because of non-additive gene action. The provision is that genetic distances between the lines to be crossed should be sufficiently large for non-additive gene action to be expressed (Pirchner, 1969).

Crossbreeding

Crossbreeding can be defined simply as the mating of unrelated parents (Dalton, 1981); more specifically it is the mating of different subspecies or distinctly different lines or breeds to each other. Breeds have been defined as a group of animals within a species, that has a common origin and certain physical characteristics that are distinguishable (Dalton, 1981). Another definition is that a breed may be considered as a Mendelian population that is differentiated from other breeds by gene frequencies (Pirchner, 1969).

Crossbreeding leads to an increase in heterozygosity, and thus benefiting fitness and the ability of animals to adapt to a wider range of environments. The crossbred will theoretically perform better than the average of the purebred parents (midparent value) for reproduction traits. The primary reasons for crossbreeding in a commercial livestock production are to utilize hybrid vigour, to take advantage of breed effects and to use breed complementarity. Hybrid vigour or heterosis will subsequently be discussed in detail, whereas breed effects and complementarity will only be discussed briefly. Breed effects involves taking advantage of the desirable traits of the parental breeds for example by crossing an African cattle breed known for hardiness with a European cattle breed known for meat quality attributes. The aim of implementing such a cross is to produce offspring that combines favourable traits from both parental lines. Complementarity is choosing

which breed to use as sire or dam. It is important to select a breed that excels in reproduction as a dam breed, whereas growth rate, production and product quality could be emphasized in a sire breed (Bourdon, 2000)

Crossbreeding systems

There are basically two crossbreeding systems; namely specific crossbreeding (which can also be called terminal crossbreeding) and continuous crossbreeding. These two systems may be adapted to suit needs and are sometimes combined (Bourdon, 2000)

A specific crossbreeding system is used when a specific type of crossbred animal is the main product of the enterprise, and all animals of this type are sold. None of these crossbreds are therefore retained for breeding purposes, but all are rather grown out to slaughter age and then slaughtered. The advantage of such a system is that complementarity can be utilised. Complementarity can be applied in a situation where a breed that exhibits poor performance in certain areas (like reproduction traits) but also possesses attributes that are cardinal to the production process (for instance excellent growth and carcass quality). Another breed may be capable of a good reproduction rate, but may perform mediocre for growth and carcass traits. The former breed would typically be used as a sire line in a specific crossbreeding system, while the latter line would be preferred as a dam line. These breeds can then be combined to produce offspring destined for slaughter in a specific crossbreeding system. The system would benefit from an improved reproduction in the dam line, as well as an improved growth rate and an increased survival of the crossbred progeny. The latter traits would typically benefit from direct heterosis. The only disadvantage of such a system is that replacement females must either be purchased in or bred in a separate operation, which would complicate management of the animals.

In contrast, any number of breeds can be used in the continuous crossbreeding system. The most important benefit of this system is that breeding females that may serve as replacements are produced. The result is that both the dams and offspring are crossbred, so heterosis on maternally influenced traits may also be exhibited. The only disadvantage is that the use of complementarity is restricted. As specific breed attributes, as well as genetic distances among breeds, are not always known, the decision of which breeds to use may not be straightforward. This leads to the system becoming more difficult to manage, with the potential of being reduced to a haphazard combination of germplasm without a well-defined objective.

Heterosis

Heterosis is the result of non-additive gene action. It is manifested in an improved performance of crossbred progeny relative to the mean performance of the breeds used to form the cross. In general, lowly heritable fitness traits are expected to benefit more from non-additive gene action, and are thus more likely to express heterosis than other production and product quality traits (Pirchner, 1969; Wilham & Pollak, 1985). Heterosis with a positive effect is referred to as hybrid vigour (Dalton, 1981). It is also sometimes referred to as the complement of inbreeding depression (Pirchner, 1969).

The cause of heterosis can be dominance or epistatic effects (Pirchner, 1969). Heterosis is absent in traits influenced only by additive gene action (Pirchner, 1969). The dominance effect of heterosis can be exhibited where favourable alleles are generally dominant over unfavourable alleles. If a homozygous line with generally favourable alleles for fitness traits is crossed to a line with mostly unfavourable alleles for fitness, the resulting offspring will be heterozygous, and the favourable dominant genes will mask the effect of the unfavourable recessives (Pirchner, 1969). This will be manifested in an improvement of fitness in the crossbred progeny relative to the parental breeds. The effect of heterosis is generally proportional to the genetic distance between the parental breeds (Pirchner, 1969).

Heterosis for a specific trait depends on three components, namely the direct, maternal and paternal component. The direct component of a trait stems from the genes of an individual on its performance, whereas the maternal component is the effect of genes inherited from both parents, but only expressed by the dam of an individual. Such genes influence the performance of the individual when it is used as a female parent or dam. Traits affected by the maternal component are typically sex-limited traits like reproduction and milk yield (Bourdon, 2000). The parental component is the effect of genes in the sire of an individual that influence the performance of the individual through the environment provided by the sire, but it is more related to the mean effect of the genes in the sire on fertility measures that are considered traits of the dam or offspring. However for the interest of this study, the focus will be on maternal heterosis exhibited in reproduction traits.

The influence of the maternal component of heterosis has been widely studied in other livestock species, and is known to influence body weight and reproductive traits (Wall *et al.*, 2005; Saadey *et al.*, 2008). Maternal heterosis influences egg production and thus the reproductive performance of the dam. Maternal heterosis of 12.3% has been estimated for annual egg production in chickens (Khalil *et al.,* 2004). Maternal heterosis for total number of eggs in Egyptian chickens ranged from -3.51% to 11.5% (Saadey *et al.*, 2008). However, no information has been published concerning the influence of maternal heterosis on reproductive traits of ostriches.

Inbreeding

Continuous breeding of purebred lines of finite size results in an increase of homozygous loci. The appearance of lethal alleles and undesirable traits may thus become more frequent under such a system (Pirchner, 1969). This leads to a reduction in fitness and an impaired reproduction rate. An increase of 10% in inbreeding depression has led to a 2.3% decrease in milk production in dairy cows (Wall *et al.*, 2005). Inbreeding is detrimental to fertility and its correlated traits in dairy cows. The SAB ostrich are often considered to be an inbred population (Jarvis, 1996). However, in contrast to the latter statement, literature has confirmed that the SAB breeds exhibits superior reproduction performance in comparison to the ZB breed (Cloete *et al.,* 2008).

Crossbreeding in the ostrich industry

Crossbreeding has been widely practiced in most mammalian and avian livestock species (Gowe & Fairfull, 1995). However, commercial ostrich producers breed the available ostrich breeds in a completely unstructured way, without any scientific base (Petite & Davis, 1999). Published studies involved the survival of crossbred chicks from a SAB and ZB cross relative to the parental breeds (Essa & Cloete, 2006; Engelbrecht *et al.,* 2008), as well as the estimation of heterosis for growth traits for the SAB and ZB cross (Engelbrecht *et al.*, 2008). Studies were also conducted to compare the reproductive performance and the meat quality and sensory properties of purebred SAB and ZB birds against that of the crossbred combination of the ZB male x the SAB female (Brand, 2006; Cloete *et al.*, 2008b; Hoffman *et al.*, 2008). Another preliminary study compared the meat quality traits of the SAB, ZB and the KR, as well as crosses of the ZB with the SAB and KR with the SAB (Davids *et al.*, 2010). These studies confirmed that the SAB breed was superior in reproduction traits compared to the ZB, whereas the ZB and KR breeds were generally superior to the SAB in terms of live weight. Most of the above studies involved only the SAB and ZB breeds and their crosses, with only one study reporting the relative performance of the KR breed in comparison with the SAB and ZB breeds (Davids *et al.,* 2010).

Ostrich Growth

Growth functions can be divided into three broad categories; i.e. models that describes diminishing returns behaviour (e.g. monomolecular); models that describe sigmoidal behaviour with a fixed inflexion point (e.g. Gompertz and Logistic), and models that describe sigmoidal behaviour with a flexible point of inflexion (e.g. Von Bertalanffy and the Richards functions) (Darmani Kuhi *et al.*, 2010). The monomolecular is the simplest nonlinear function. Growth is constant and independent of the weight of the organisms in this case. It works at a rate proportional to the substrate level (nutrient varies depending on the species) and growth is irreversible (France *et al.*, 1996). The Logistic function can be derived by assuming that the quantity of growth is proportional to body weight. The inflexion point is fixed at exactly the half of the theoretical final body weight (France *et al.*, 1996; Darmani Kuhi *et al.*, 2010). For the Gompertz function, it is assumed that the nutrient supply is non-limiting; the quantity of growth is proportional to body weight, and the effectiveness of growth decays exponentially with time according to a constant. For the Von Bertalanffy function, the assumption being made is that the nutrients are non-limiting, and that the growth process is the difference between anabolism and catabolism. It has a flexible inflexion point. The Richards function is more empirical, and does not have the same underlying biological basis as the Von Bertalanffy function. Its flexibility is due to its shape parameter *n*, which is dimensionless. The inflexion point can be reached at any fraction of the mature weight as n varies over a range from -1 to infinity (France *et al.*, 1996; Darmani Kuhi *et al.*, 2010).

Non-linear models like the Gompertz, Richards function, Logistic function, and spline regressions were all used to measure growth against time in poultry (Mignon-Grasteau *et al*., 1999; Mignon-Grasteau *et al.,* 2000; Aggrey, 2002; Goliomytis *et al*., 2003; Sakomura *et al*., 2005; Norris *et al.*, 2007). However, the most popular growth model used in poultry is the Gompertz equation. Comparative studies executed between models (Richards, Logistic function and a spline regression model) reported that the Gompertz and Richards

function was the most suitable for growth in chickens. (Aggrey 2002; Norris *et al.*, 2007) The Gompertz function is the only growth function that has been most accurately fitted to ostrich growth curves (Du Preez *et al.*, 1992; Cilliers *et al.*, 1995).

There are different forms of the Gompertz equation. However, the three parameters of concern are; A which can be described as the adult value or asymptotic limit of the weight when age reaches infinity (t), B, the rate of growth and C, the age of maximum weight gain in days. The Gompertz function is known to have a fixed inflection point, which is related to the maximum asymptotic body weight (Darmani Kuhi *et al.*, 2010). This inflection point is the point at which no further increase in growth occurs, and a reduction in the slope of the growth curve is observed. This pattern of growth is typical in mammals and avian species (Emmans, 1981; Emmans, 1988).

Little information is available on the growth curves of the different ostrich breeds and their crosses currently farmed with in South Africa. The information available illustrates that there are differences in the growth as reflected by mature live weight of the three dominant ostrich breeds for commercial production, namely the SAB, ZB and KR (Jarvis, 1998). However, no significant differences were observed for the mature live weight (A-parameter) between SAB, ZB and Namibian ostriches (Du Preez *et al.* 1992). The SAB displayed the slowest growth of the three breeds, with the ZB exhibiting more rapid growth (Du Preez *et al.,* 1992). No studies have been performed on the growth rate of the KR breed.

The ideal of producers of slaughter animals is to have a breed that reaches a slaughter weight after the shortest possible growth interval. This will lead to reduced input costs, regarding feed and other maintenance costs of the bird and in turn lead to a more effective production system. Therefore, birds for slaughter must be processed closer to inflection points to decrease production costs (Navarro *et al.,* 2005). It is known that the maximum growth of an animal occurs at about one third of the interval before maturity is reached (Cilliers *et al.*, 1995). The expected ages of physiological maturity have been estimated to be 602 to 664 days for SAB ostriches (Cilliers *et al.,* 1995). Conventionally ostriches have been slaughtered at an age of approximately 14 months. Economic considerations necessitated the slaughter age of ostriches to be reduced markedly in the recent past (Bhiya, 2006). It would thus be beneficial to produce a breed that reaches a heavier slaughter weight (with an acceptable meat and skin quality) at slaughter ages younger than 14 months.

It can be assumed that the quickest genetic solution to the achievement of an increased slaughter weight after a shorter growth period would firstly be to substitute the SAB (with a mediocre growth performance) with a faster growing breed like the ZB or the KR. To make an informed decision in this regard, the possible penalties of breed substitution on production and reproduction traits needs to be understood. Alternatively, crossing the relative slow-growing SAB breed with breeds with an inherently higher live weight which is expected to be capable of achieving faster daily gains could be contemplated. This study therefore incorporates a chapter on this topic to increase the present knowledge of growth of the breeds forming part of the genetic resource available to the local industry.

Profile of Ostrich meat

Ostrich meat is regarded as an intermediate type of red meat, which is classified as a red muscle tissue type (Sales, 1996). Its dark red appearance is due to a high myoglobin content ranging from 5.10 mg/g^{-1} to 9.1 mg/g^{-1} meat depending on the specific muscle (Sales, 1996). Ostrich meat contains higher myoglobin concentrations than other red meat species such as cattle and sheep. The myoglobin content is known to vary between different wildlife species, such as the oryx, kongoni and zebra (Onyango *et al.*, 1998), but generally the meat of free-ranging or active animals contains higher myoglobin concentrations than that of sedentary animals (Warriss, 2000).

Juiciness in meat is predominantly related to the amount of intramuscular fat (Lawrie & Ledward, 2006). Ostrich meat is known to have a low intramuscular fat content (Sales, 1996), and has been observed to have a drier taste than beef steak (Harris *et al.,* 1994). Optimal meat tenderness is associated with a rate of muscle pH decline, whereby a pH of 5.9 to 6.0 is reached three hours post-mortem (Lawrie & Ledward, 2006). Ostrich meat has a rapid pH decline until two hours post-mortem for the *ambiens* and *iliofibularis* muscles (Sales & Mellet, 1996), and reaches an ultimate pH in the range between 5.8 and 6.2 as reported by Sales (1994) and 5.83 ± 0.09 by Botha *et al.* (2007). Emu meat reaches a mean ultimate pH of 5.5 within 3 hours post-mortem (Berge *et al.,* 1997). The M.*iliofibularis* of the ostrich was reported to be similar in tenderness and taste to beef loin steak (Harris *et al.,* 1994). The M.*iliofibularis*, also more commonly known as the fan fillet is the heaviest ostrich steak (Sales, 1996) and because of its popularity, is the steak that is mostly used as a representative of all the other muscles in scientific research.

Pre-slaughter stress also has an influence on the pH due to the secretion of adrenalin during the occurrence of stress. Adrenaline activates the enzyme phosphorylase which breaks down glycogen to glucose-1-phosphate (Lawrie, 1998). Therefore, the glycogen reserves of stressed animals pre-slaughter are depleted more rapidly than those of docile animals. This results in a higher ultimate pH of stressed animals compared to calm animals because the accumulation of lactic acid due to anaerobic glycogenolysis are reduced (Lawrie, 1998).

The ultimate pH of meat is negatively correlated with the water holding capacity (WHC) of meat (Lawrie, 1998). Drip loss and cooking loss percentages are indicators of the WHC of meat. Water holding capacity is at a minimum at a low ultimate pH (Onyango *et al.*, 1998). This phenomenon can be explained by the role of pH in the breaking down of the protein structures of the meat. A low pH is resultant of an acidic environment, which leads to the breakdown of protein structures and resultant water loss. Therefore, the rate and extent of muscle acidification may affect the amount of moisture exuded as drip loss. Meat with a high drip loss percentage has a poor WHC. Ostrich meat generally has a high WHC, which is favourable for meat used for processing (Lawrie, 1998). However, a high WHC of meat is adversely related to shelf life (Lawrie, 1998).

Ostrich meat has a relatively high moisture content (76.59%), a low collagen tissue concentration (0.429 g/ 100g muscle), low saturated fatty acid concentration (35.65%) and a relatively high poly-unsaturated fatty acid concentration (31.75%) (Sales, 1994). The cholesterol content (62.42 mg/100g meat) of ostrich meat is similar to that of other slaughter animals (Sales, 1994). The percentage of total saturated, mono-unsaturated and poly-unsaturated fatty acids is relatively constant between all muscles in the ostrich (Sales, 1998). The fatty acids with the highest concentration in ostrich muscle are oleic acid (C18:1n-9) and palmitic acid (C16:0), followed by linoleic acid (C18:2n-6) and stearic acid (C18:0) (Hoffman *et al.*, 2005). Hoffman *et al.* (2005) noted that saturated fatty acids were 40% in muscle and 50% in the abdominal fat reserves.

However, with all the information already available for ostrich meat, few studies have compared the meat quantitative and qualitative traits of the breeds available in South Africa (SAB, ZB and KR) as well as their crosses. Studies that have been conducted includes carcass and muscle yields of ostriches as influenced by breed (Hoffman *et al.*, 2007) as well as the influence of breed on the physical and sensory meat quality traits (Hoffman *et al.*, 2008). For the same aged birds, the SAB breed had lower live carcass and leg weights in comparison with the ZB while the crossbred birds (ZB males mated to SAB females) resembled the ZB for these weight traits (Hoffman *et al.*, 2007). The ZB had a higher pH 24 hours post mortem than the SAB, but there were no significant breed differences as pertaining to the sensory characteristics of the meat (Hoffman *et al.*, 2008). The major weakness of the two studies cited here is that a small sample size of only two birds represented the ZB breed. Because of this, chance could have resulted in some of the observed differences, although significance could be demonstrated. A reciprocal cross was absent in both studies and therefore the effect of heterosis on meat traits could not be estimated accurately. In addition few studies in the literature investigated the meat traits of the KR breed, either as a pure breed, or when crossed with the SAB. A study reported by Davids *et al.* (2010) shows that the KR x SAB cross resembled the SAB in terms of meat quality traits and the KR in terms of live weight, as was previously found in studies on the ZB. The KR was also reported to have a higher final pH than the SAB (Davids *et al.,* 2010). Information on the meat quality of the KR is necessary as these breeds are crossed with the SAB without knowledge of their impact on meat quality traits (Petite & Davis, 1999).

It is clear that although some effort has gone into the study of meat attributes of the SAB and ZB breeds as well as of the ZB x SAB cross, that many issues remains unresolved. No studies on the meat quality of the KR and crosses derived from this breed were found in the literature. Part of the focus of this study thus focuses on meat quality of the SAB, ZB and KR. The expansion of the available data to include crosses of the ZB and KR with the SAB also contributed to an opportunity to confirm or refute some findings stemming from previous publications.

Reproduction

Reproduction could possibly be regarded as one of the key traits determining the economic viability of the ostrich industry (Cloete *et al.,* 2002, 2008a). The reproduction of SAB and ZB birds differed appreciably in the favour of the SAB breed (Brand, 2006; Cloete *et al.,* 2008b). It needs to be stated that the breed comparisons that were cited relied on a largely unquantified ZB resource in terms of pedigree information

and age. It is therefore important to expand the study of Cloete *et al.* (2008) to including ZB birds with a known ancestry, being maintained in a flock with a known age structure. In view of the previous discussion, the highly reproductive SAB breed could thus be considered as a suitable dam line in specific crossbreeding systems. In contrast, the ZB males show less potential in this respect. No published reports of reproduction of the KR could be found in literature.

Crossbred female farm animals are commonly used as the dam line in specific crosses with meat-type sire lines (Kress *et al.,* 1990). Such systems aim to make optimal use of heterosis in the crossbred animals. Heterosis is expected in fitness traits such as reproduction. So far, no structured studies have been conducted on the crossbred ostrich female as a dam line in terminal crossbreeding operations for commercial production. It is thus needed to confirm or refute the results of previous studies on the reproduction of purebred SAB and ZB females. Comparative studies should also include the KR breed for which no figures are available at present. This study also seeks to address this shortcoming.

In general, the literature reviewed above reported on breed effects for reproduction, chick growth and survival, as well as or slaughter traits (Brand *et al.,* 2005; Hoffman *et al.,* 2007; Cloete *et al.,* 2008b; Engelbrecht *et al.,* 2008). In some of these studies, crossbreeding has also been done and heterosis were observed for traits like slaughter weight and chick survival (Essa & Cloete, 2006; Engelbrecht *et al.*, 2008). Phenotypic differences in live weight have also been observed between these three breeds (Jarvis, 1998). These studies all seem to suggest that reproduction, growth and meat traits differ substantially between the SAB, ZB and KR, in other words, that the genetic distances between them would be suitably large to ensure heterosis. However, the differences have not been substantiated in molecular studies on the South African ostrich breeds. Genetic markers, especially microsatellites markers, are popular in determining genetic differentiation between and within populations. Microsatellites and its application in local ostrich breeding will therefore be discussed in the following section.

Microsatellites

Microsatellites are also known as short tandem repeats (STR's) (Avise, 2004), and represent a tandem repeat of di, tri or tetra nucleotides (Avise, 2004; Edwards & McCouch, 2007). These sequences may also be referred to as simple sequence repeats (SSR) or sequence-tagged microsatellite sites (STMS) (Van Marle-Koster & Nel, 2003). These nucleotides can mostly be mapped to precise positions in the genome. If the number of repeats differs at such a specific position in an individual, a microsatellite can be used as a genetic marker for usage in linkage disequilibrium studies. The most commonly found microsatellites in eukaryotic cells involve the CA and TA repeats. These repeats are frequent and it covers the whole genome. It is highly polymorphic due to the variation in repeats (Van Marle-Koster & Nel, 2003). This variation might be due to slipped strand mispairing causing frequent gain or loss of repeats (Edwards & McCouch, 2007).

Because of their high level of allelic diversity, microsatellites are valuable molecular markers in studies on closely related individuals (Edwards & McCouch, 2007). Polymerase chain reaction (PCR) based markers are designed to amplify fragments that contain a microsatellite using primers complementary to the unique

sequences surrounding the repeat motif (Edwards & McCouch, 2007). Microsatellites discovered in the non-coding region, have a higher rate of polymorphism than microsatellites in genes (Edwards & McCouch, 2007). Microsatellites are co-dominant markers, thus the heterozygote can be discerned from the homozygous state (Edwards & McCouch, 2007). It can be easily automated using fluorescent primers on an automated sequencer and it is possible to multiplex several markers with non-overlapping size ranges on a single electrophoresis run (Edwards & McCouch, 2007).

Benefits of microsatellites are that their results are reproducible, and markers are easily shared among researchers simply by distributing primer sequences (Edwards & McCouch, 2007). However, uneven distribution of microsatellites across the genome limits their usefulness (Edwards & McCouch, 2007). Microsatellites tend to mutate with mutation rate of up to 10^{-2} per generation (Bruford & Wayne, 1993), therefore containing variation within a population (Avise, 2004). This rapid mutation rate results in alleles that may be identical in size, but not necessarily identical by descent. This can lead to difficulty during the interpretation of microsatellite results, especially for population structures that are separated geographically (Avise, 2004).

Application of genetic markers in genetic diversity studies in ostriches

The application of molecular markers and microsatellites in particular, for genetic diversity studies in poultry and livestock species has been well established throughout the literature (Muchadeyi 2007; Mtileni *et al.*, 2010; Li *et al*., 2004; Zhou *et al*., 2005; Vicente *et al*., 2008). Measurements that are estimated to gain insight in genetic diversity between and within breeds or breeds include the average observed and expected heterozygosity, the polymorphic information content and number of alleles by locus. Because microsatellites are highly polymorphic, they have become popular as markers for use in studies on genetic diversity. Other molecular markers have also been used to investigate genetic diversity in ostrich populations. These markers include restricted fragment length polymorphisms (RFLP) (Freitag & Robinson, 1993) as well as DNA fingerprinting (Kawka *et al.*, 2007).

Genetic differences have been found between the KR and the Somalian blue ostrich (Kumari & Kemp, 1998) as well as between the SAB, ZB and KR (Kawka *et al.*, 2007). These studies involved small ostrich populations in the UK in the case of the former group, and in Poland in case of the latter group. There have also been studies in Africa on the molecular genetic relationships based on mitochondrial DNA (mtDNA) with RFLP genetic markers between the five ostrich subspecies (Robinson & Matthee, 1999). These results suggested that the Arabian ostrich (*S. c. syriacus*) shared maternal ancestral lineage with the Northern African ostrich (*S. c. camelus*) and that the Southern ostrich (*S. c. australis*) are in close relationship with the Kenyan ostrich (*S. c. massaicus*) (Robinson & Matthee, 1999). However, no studies have been done on the genetic diversity between and within the breeds comprising the ostrich genetic resource in South Africa. A low genetic diversity has been reported for the *S. c. australis* under which domestic ostriches from the Klein Karoo in South Africa were classified at that stage (Robinson & Matthee, 1999).

Other applications of microsatellites in ostrich breeding

Microsatellites have also been used for the sexual identification of ostrich chicks during the first days of nestling (Malogó Jr *et al.*, 2002). Microsatellites were also utilized to identify the parentage of ostrich chicks in order to resolve the challenge of pedigree recording in the communal nesting system (Essa, 2005). A study was done also to determine the sequence homology between chicken microsatellites and the genome of the ostrich, which showed that the microsatellites used for mapping in the chicken genome cannot be applied directly to ostriches (Horbanczuk *et al.,* 2007). A preliminary ostrich linkage map has been constructed using 118 microsatellite markers (Huang *et al.,* 2008). This map is 440.3 cM in length. Twelve linkage groups containing 41 markers were constructed, belonging to 12 autosomes.

Framework of the study

To conclude, it is reiterated that the different breeds of ostriches important to the South African ostrich industry include the SAB (*S. c. domesticus*); ZB (*S. c. australis*) and the KR (*S. c. massaicus*). Preliminary studies that were reviewed suggest marked differences between the SAB and ZB breeds for reproduction, growth and slaughter traits. From this it can be inferred that genetic distances between these breeds would be sufficiently large to allow the expression of heterosis. Information on the KR breed performance is still insufficient and more information needs to be obtained. Similarly, results on crossbreeding and possible heterosis of ostrich breeds are scant. This study aims to confirm preliminary results reported previously, and to add to existing information on production of the KR breed and crosses between the SAB and ZB breeds. Furthermore, the genetic variation between and within these breeds have not yet been quantified on a molecular level in South Africa. This study also seeks to address this shortcoming using microsatellite markers to determine the genetic distances between the three breeds (SAB, ZB and KR). In combination, the separate studies are seen to guide the South African ostrich industry as pertaining to the commercial exploitation of breed diversity and hybrid vigour.

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Chapter 3

Growth curves of purebred South African Black, Zimbabwean Blue and Kenyan Redneck ostriches, as well as the reciprocal cross between the former lines

Abstract

Weight records at monthly intervals were obtained for purebred South African Black (SAB), Zimbabwean Blue (ZB) and the Kenyan Redneck (KR) ostriches, as well as for the reciprocal cross between the SAB and ZB. Individual Gompertz non-linear regressions were fitted to the data of 390 purebred and 41 crossbred ostriches, using the SAS NLIN function In case of the SAB and ZB breeds and their reciprocal crosses, heterosis was estimated for each parameter of the Gompertz model. The estimated adult weight (A-parameter) of the ZB (147 kg) and the KR breeds (148 kg) were higher than that of the SAB breed (129 kg). The overall growth rate (B-parameter) of the ZB breed (0.0075) and the SAB breed (0.0080) was lower than that of the KR (0.0150). The age at maximum weight gain (C-parameter) was higher for the ZB breed (226 days) compared to the SAB (198 days) and the KR (194 days). Heterosis for the A-parameter was estimated at -6.2% and at -12% for the C-parameter. A tendency towards heterosis for the C-parameter was supported by significant heterosis estimates in live weight at 12 months (6.9%) and 15 months (4.0%). Results for the purebreds suggest that the ZB breed may grow out to a higher mature weight than the SAB in particular, but that the former breed is later maturing than the other breeds. Further studies are needed to adapt the application of crossbreeding among the breeds constituting the present South African ostrich genetic resource.

Introduction

The ideal of any slaughter bird producer is to have a breed that reaches a high slaughter weight gain after the shortest possible growth period. This will lead to overall lowered input costs, regarding feed and other husbandry costs of the bird and therefore a more effective and profitable production system. Total costs are known to increase with slaughter age (Bhiya, 2006). In general, slaughter birds must be processed closer to inflection points to decrease production costs (Navarro *et al.,* 2005). It is known that the maximum growth of an animal occurs at one third of the age before sexual maturity (Cilliers *et al.*, 1995). The expected ages at maturity have been estimated at between 602 and 664 days for SAB ostriches (Cilliers *et al.,* 1995). Traditionally ostriches have been slaughtered at an age of approximately 14 months. Weights for different breed combinations at this stage were respectively 89.6, 98.3, 100.8 and 99.6 kg for the SAB, ZB, SAB x ZB, ZB x SAB breeds respectively (Engelbrecht *et al.*, 2008). However, with restructuring in the cost and product price structures, slaughtering of birds at 10.5 months were shown to lead to the highest margin above costs (Bhiya, 2006). Thus it would be beneficial to utilize a breed that reaches a heavier slaughter weight over a shorter period of time.

Breed differences are evident between the SAB, ZB and KR in terms of mature live weight, in favour of the latter two breeds (Jarvis, 1998). In contrast, the SAB has been demonstrated to have higher chick production (measure of reproduction performance) when compared to the ZB and cross combinations (Brand *et al.*,

2005; Cloete *et al.*, 2008) and also more favourable meat quality traits compared to the ZB (Davids *et al.,* 2010). The SAB breed acquires a substantially lower live weight compared to its contemporaries, as noted above. Crossbreeding is known to make use of complementarity, and allows the commercial exploitation of hybrid vigour (heterosis) for traits like early growth and survival.

There are different forms of the Gompertz equation mostly used for the description of growth. However the three parameters of concern are the A-parameter which can be described as the adult value or asymptotic limit of the weight when age reaches infinity (defined as t), the B-parameter, representing the rate of growth and the C-parameter which represents the age of maximum weight gain in days. The Gompertz function is known to have a fixed inflection point which is related to the maximum asymptotic body weight (Darmani Kuhi *et al.*, 2010). This inflection point is the point at which no further acceleration of growth occurs and a reduction in the slope is observed. This pattern of growth is typical in mammals and birds. The Gompertz equation has been successfully applied for growth analyses of various poultry species (Mignon-Grasteau *et al*., 1999; 2000; Norris *et al.,* 2007). The growth curve of ostriches is also known to be sigmoid and asymptotic and therefore the Gompertz function has been successfully fitted to ostrich growth data of this species as well (Du Preez *et al.*, 1992; Cilliers *et al.*, 1995, Sabbioni *et al.*, 1999). Little information is available on the specific growth curves of the different ostrich breeds that are currently farmed with in South Africa, as well as crosses between these breeds.

Theoretically increased hybrid vigour is obtained when the breeds crossed are genetically more distant from each other. The genetic distance is greatest between the KR and SAB, while the SAB and ZB are somewhat closely related to each other (Freitag & Robinson, 1993). Crossbreeding of these three breeds often occurs randomly without any proper planning (Petite & Davis, 1999). Thus it is important to estimate crossbreeding parameters that will aid in defining well structured crossbreeding systems in the ostrich industry.

Therefore the aim of this study is to determine if there are any significant differences in the growth curves of the breeds mentioned previously, as well as the reciprocal crosses between the SAB and ZB. Heterosis for growth traits and parameters of the Gompertz growth curve will also be estimated.

Material and methods

Animals and management

Slaughter birds were grown out on the Oudtshoorn Research Farm during the period from 2007 to 2009. After hatching as described by Brand *et al.* (2008) the chicks were transferred to a chick house. The temperature of the chick house were 26 °C for the first week, whereafter the temperature were decreased by 1°C at weekly intervals. At three weeks of age the chicks were familiarised to the outside environment. The chicks were moved to bigger feedlots at four weeks of age, where they walked and were fed outside during the day but were provided housing during the evening. For the first 30 days they received bio-vitamins mixed with luke-warm water every morning, to strengthen their immune systems. They also received a balanced

pre-starter diet for the first ten days that were sprinkled with chopped lucerne and oats. From three months of age the chicks were left outside depending on the ambient conditions. At the age of three to four months the chicks were moved to the feedlots where they received a grower diet of 10.5MJ/ME energy and 160g protein/kg dry matter on an *ad libitum* basis. Water was made available *ad libitum* and the water containers were cleaned and disinfected at regular intervals. The juveniles were sorted according to their weight to ensure that they received optimal feeding and were weighed monthly (Engelbrecht *et al.,* 2008)

Growth data were acquired from three purebred ostrich breeds namely the SAB, ZB and the Kenyan Redneck (KR) as well as crosses between the former breeds (SAB male x ZB female; ZB male x SAB female).The original data set recorded consisted of 11685 individual monthly weight records which was accessed from the Oudtshoorn Research Farm. After editing (deleting records of individuals that had less than four records or other missing records) 2228 SAB, 202 ZB, 93 KR, 162 SAB x ZB and 101 ZB x SAB repeated weight records were used to construct individual Gompertz growth patterns. These weights were recorded for chicks hatched during 2007 to 2008 when all the possible breed combinations were available and represented by a reasonable number of weights.

All the birds were weighed at the following approximate intervals: Day after hatching (one day), and at approximately one month, three months, six months, nine months, 12 months and 15 months of age. Individual values for the parameters A, B, C could thus be derived for all the birds in the analysis.

Statistical analysis

The growth of the different breeds was fitted to the Gompertz equation using the NLIN procedure for the estimation of nonlinear regression parameters (SAS, 2006). A goodness of fit test was run comparing the GLM (specifying no intercept) SSQ to the SSQ of the non-linear (NLIN). The NLIN model for growth fitted the data best, as follows:

Gompertz function for individual growth = A*(exp (-exp (-B*(AGE-C))))

Where:
A= the adult weight or asymptotic limit of the weight when age (AGE) approaches infinity.
B= overall rate of growth
C= age at maximum weight gain in days

Thereafter, the general linear model (GLM) procedure of SAS (2006) was used to analyse growth parameters of individual birds to test for the following fixed effects: Breed of the individual, year, month of hatch and gender. Weights at monthly intervals for the various breeds were also fitted against the breed of the individual, year, month of hatch and gender in a subsequent analysis. The model applied was:

$Y_{ijk} = \mu + S_i + Y_j + M_k + \epsilon_{ijkl}$

Where:

Y_{ijk} = the l^{th} live weight observation of the i^{th} breed in the j^{th} year and the k^{th} month of hatch

μ = the overall mean

S_i = the fixed effect of the i^{th} breed (breed)

Y_i = the fixed effect of the j^{th} year

M_k = the fixed effect of the k^{th} month of hatch

ϵ_{ijkl} = residual variance

The differences between the means for these observations were estimated using post hoc analysis employing the Bonferonni method (SAS, 2006).

Results and Discussion

Performance of pure breeds

According to Table 3.1, the final mature live weight (A-parameter) of the ZB and KR breeds were respectively 12% and 13% higher ($P < 0.05$) than that of the SAB breed. These differences are comparable with those reported previously for the difference in live weight between the SAB and ZB breeds (Jarvis, 1998; Brand, 2006; Cloete *et al.,* 2008) and the SAB and KR breeds (Jarvis, 1998). The absolute value for estimated maximum adult weight of the SAB (129 ± 1.8 kg) deviated slightly from results for reproducing males published by Brand (2006) and Cloete *et al.* (2008). Mean live weights of 122 ± 2 kg and 123 ± 1kg were respectively reported for mature SAB ostrich males at the commencement of breeding in these studies. However the adult weight estimated for the ZB breed in this study (147 ± 4.1 kg) was higher than values of reported by Brand (2006) and Cloete *et al.* (2008) respectively estimated as 133 ± 2 kg and 134 ± 3 kg.

The KR breed was estimated to reach a maximum adult asymptotic weight of 148 ± 31 kg, which did not differ from the corresponding value derived for the ZB. No comparable results for the latter breed were obtained from literature. The only references reporting means for all three breeds were those of Jarvis (1998 – mature weights) and Davids *et al*. (2010 – slaughter weights). Means for the mature live weight of the SAB, ZB and KR breeds were respectively 115, 125 and 135 kg in the former study and 85, 94 and 89 kg in the latter study. The present results are in contradiction to those of Jarvis (1998), indicating that the mature KR birds ought to be the heaviest of the three breeds. However, it is in closer correspondence with those results of Davids *et al.* (2010), using the same resource population that was used in this study. The study of Jarvis (1998) was based on adult birds that was shot or slaughtered at different locations. It is thus conceivable that the derived means from the study of Jarvis (1998) could be less accurate.

The SAB and KR breeds differed significantly for their B-parameters (indicative of the rate of growth), while the overall growth rate of the ZB were similar to that the SAB breed. It is notable that the KR breed reached

a higher adult weight than the SAB but achieved maximum growth at approximately the same chronological age (198 vs. 194 days), as can be seen in Table 3.1. The large standard error is due to the relatively small number of observation on the KR breed in this study. The ZB reached a maximum adult weight comparable to that of the KR, but peak growth was attained at a later stage (226 days). These results suggest that the ZB is a heavier breed compared to the SAB, but the ZB seems to be later maturing then the other two breeds. The relative growth curves of the three pure breeds are depicted in Figure 3.1, for the age interval over which weights were recorded. The decline in growth rate towards the end of the recording period in the SAB and KR breeds is evident. The growth rate of the ZB breed, in contrast was much less affected at the end of the recording period.

Table 3.1 Means (± SE) for growth parameters of purebred ostrich breeds belonging to the South African Black (SAB), Zimbabwean Blue (ZB) and Kenyan Redneck (KR) breeds.

Parameters	Breed		
	SAB (n=342)	ZB (n=33)	KR (n=15)
A	129^{a}±1.8	147^{b}±4.1	148^{ab}±31
B	0.00797^{a}±0.00012	0.00747^{a}±0.000514	0.0150^{b}± 0.0039
C	198^{a}±2.9	226^{b}±8.4	194^{a}±37

[a-b] Row means with common superscripts do not differ (P > 0.05)

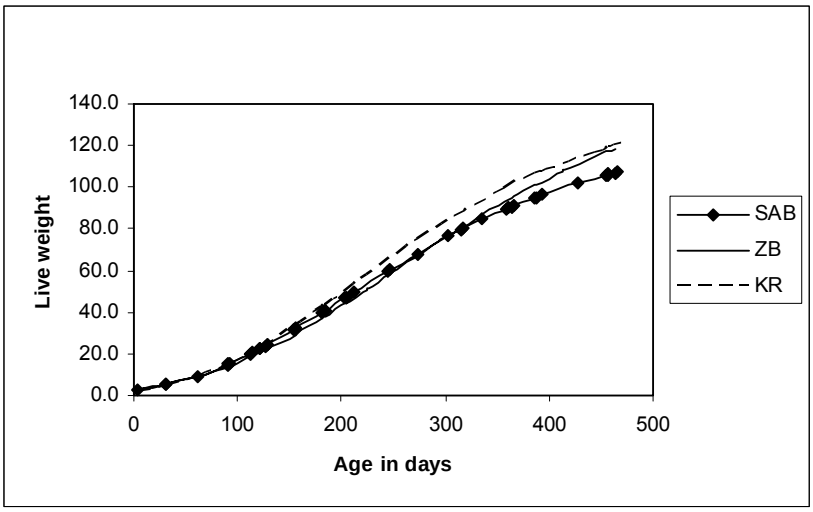

Figure 3.1 Gompertz growth curve comparing growth of the purebred South African Black (SAB), Zimbabwean Blue (ZB) and Kenyan Redneck (KR) breeds.

As pertaining to the parameters of the Gompertz curve, the overall growth parameters of the SAB and ZB in the present study were compared with the previous studies reporting comparable parameters (Table 3.2). It is notable that the mature weight (A-parameter) of SAB birds in the present study (129 kg) is about 10 kg heavier than the corresponding values reported by Cilliers *et al.* (1995) and Kritzinger (unpublished), and ≥20 kg heavier than the estimates of Du Preez *et al.* (1992) and Sabbioni *et al.* (1999). However, these high

estimates for mature live weight does seem less accurate when compared to the actual means for mature male weight in the studies of Brand (2006 – 122 kg) and Cloete *et al.* (2008 – 123 kg). The present result pertaining to the B-parameter (growth rate) for the SAB breed (0.008) is intermediate in a range of values from 0.006 to 0.01. The value for the C-parameter (198 days), depicting the chronological age when maximum growth is attained, is intermediate in a range of 156 to 257 days. It is reasonable to assume differences in nutritional management and husbandry may have contributed to the differences between studies, while changes in the genetic make-up of the SAB breed could also have contributed to differences with earlier studies of Du Preez *et al.* (1992), Cilliers *et al.* (1995) and Sabbioni *et al.* (1999).

Table 3.2 Comparison of Gompertz growth parameters (estimate ± SE) for the South African Black (SAB) and Zimbabwean Blue (ZB) breeds derived from literature sources and from the present study.

	A-parameter		B-parameter		C-parameter	
Breed	SAB	ZB	SAB	ZB	SAB	ZB
References						
Du Preez *et al.* (1992) SAB males (n = 16) & ZB males (n = 24)	102±3.7	94.2±4.6	0.0097±0.0004	0.0168±0.001	163	92
Cilliers *et al.* (1995) SAB males (n = 26)	119±2.5		0.0091±0.0003		180±6.2	
Sabioni *et al,* (1999) SAB (n = 63) & ZB (n=88)	109±4.3	111±3.7	0.00551±0.0004	0.00562±0.0004	257±5.7	246±9.6
Kritzinger (2010) (unpublished) SAB (n = 52)	119		0.0090		156	
Present study SAB (n = 342) & ZB (n = 33)	129±1.8	147±4.1	0.00797±0.0001	0.00747±0.0005	198±2.9	226±8.4

The ZB birds in the present study surpassed the performance of the comparable breed in the study by Sabbioni *et al.* (1999) for all parameters of the Gompertz curve. The performance of the ZB birds used in the latter study was also much closer to that of their SAB contemporaries than those in the present study. It is notable that the energy concentration of diets used by Sabbioni *et al.* (1999) for birds older than four months was 7.96 MJ/kg feed, which is lower than the energy level used in the present study. Moreover, the diet provided by Sabbioni *et al.* (1999) consisted of an unknown quality of lucerne hay or grass of which the quality. The difference of 14% in favour of the mature live weight (A-parameter) of the ZB birds in the present study relative to that of the SAB is supported by comparable breed differences of ~9% in mature live weight of males in the studies of Brand (2006) and Cloete *et al.* (2008). Viewed against these results, the <2% breed difference for mature live weight reported by Sabbioni *et al.* (2008) seems dubious. When the ZB birds used by Du Preez *et al.* (1992) were considered it is clear that the growth curve estimated by the latter

authors differed completely from that in the present study. Evidence from the literature cited above seems to contradict the finding of Du Preez *et al.* (1992) that the absolute mature weight (A-parameter) of the ZB birds should be lower than that of the SAB breed.

Crosses between the SAB and ZB

The results reported above suggest that crossing the SAB breed (low adult weight but earlier maturing) with the ZB and KR (higher adult weight, but slower to mature in the case of the ZB) can result in a faster-growing bird capable of attaining a higher live weight at slaughter age. The effect of crossing the SAB and ZB breeds (where reciprocal crosses were possible) was assessed and is described next. As the relative performance of the SAB and ZB breeds has been described, more emphasis will be placed on the performance of the crosses in the following section.

As pertaining to the purebred SAB and ZB breeds and their reciprocal cross, there were significant differences between the breeds for the respective growth parameters of the Gompertz model. Asymptotic adult weight (the A-parameter) of the SAB differed from the purebred ZB breed (Table 3.3). The ZB x SAB cross was estimated to reach a similar asymptotic adult weight as ZB birds, with the reciprocal cross being estimated to be lighter than the SAB. Growth (the Gompertz B-parameter) was also affected by breed, being similar for the purebred breeds (SAB and ZB), higher in the SAB x ZB breed, and lower in the ZB x SAB breed ($P < 0.05$). In order of the relative magnitude, the means for the age at which maximum growth was attained (C-parameter) resembled those of the A-parameter, with the ZB x SAB breed resembling the purebred ZB breed and the SAB x ZB breed reaching maximum growth earlier than the purebred SAB breed (Table 3.3). From the plotted graphs it was evident that the ZB breed grew slowly at first (Figure 3.2) but exhibited an increase in growth rate at a later stage. This might possibly be ascribed to the ZB struggling to adapt to the environment imposed on them by artificial chick rearing, as was speculated by Engelbrecht *et al.* (2008).

Table 3.3 Means (± SE) for growth parameters of purebred South African Black (SAB) and Zimbabwean Blue (ZB) breeds and the reciprocal cross between the SAB and ZB breeds.

Sire line	SAB		ZB	
Dam line	SAB (n=342)	ZB (n=16)	SAB (n=25)	ZB (n=33)
Parameters				
A	129^{b}±1.8	115^{a}±8.0	144^{c}±5.8	147^{c}±4.1
B	0.00797^{b}±0.00012	0.010^{c}±0.00079	0.00673^{a}±0.000296	0.00747^{b}±0.000514
C	198^{b}±2.9	158^{a}±12	214^{c}±9.8	226^{c}±8.4

[a-c] Row means with common superscripts do not differ ($P > 0.05$)

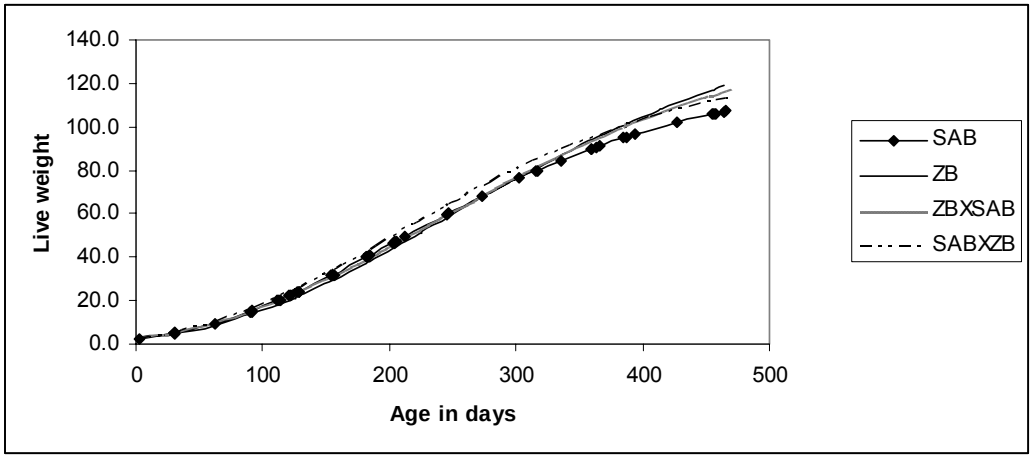

Figure 3.2 Gompertz growth curve of the purebred South African Black (SAB) and Zimbabwean Blue (ZB) breeds, as well as the reciprocal cross between these breeds.

Significant negative heterosis estimates were obtained for the SAB and ZB cross for the A and the C parameters, while heterosis estimated for the B-parameter also approached significance (P = 0.08). An estimate of 8.4% for the B-parameter suggest that growth rate of crosses between the SAB and ZB breeds may benefit from hybrid vigour at the 92% confidence level. The results pertaining to the C-parameter suggest that the period of maximum growth in the SAB x ZB crossbred breed in particularly is reduced, possibly hinting at an improved maturity type in these crossbred birds.

Table 3.4 Heterosis (P-value) for the Gompertz growth parameters.

Parameters	SAB x ZB CROSS
A	-6.2% (0.04)
B	8.4% (0.08)
C	-12% (0.001)

Conclusions

The effect of breed of the individual was significant for all the growth parameters A, B and C of the Gompertz curve. In contrast, these parameters were independent of the year, month of hatching as well as the gender of the animal. The growth curves thus seem fairly robust and largely unaffected by these sources of variation. Differences were observed for the Gompertz growth parameters of purebred SAB, ZB and KR birds. Generally, the ZB and SAB birds maintained a slower growth rate than the KR breed. However, maximum growth of ZB birds occurred at a later age than that of their SAB and KR contemporaries, indicating that they may be later maturing than the other breeds. This allowed the ZB to grow out to a higher final live weight than the SAB, while being comparable in mature weight to the KR.

Information on the reciprocal cross between the SAB and ZB breeds allowed the estimation of heterosis for the Gompertz growth parameters. The performance of the two crossbred breeds was not consistent, thus complicating recommendations based on these results. This inconsistency may be related to relatively few animals being represented in the crossbred breeds.

The practical application of the tendency towards significant heterosis for growth (B-parameter) needs to be developed further for possible application in commercial ostrich production. Using monthly weights, significant ($P < 0.07$) heterosis estimates (%) for live weights as an indication of growth, were obtained at 12 months (6.9%) and 15 months (4.0%) of age. Details of these estimates are not provided, as it is consistent with a previous estimate of 6.7% heterosis for live weight at a comparable age (422 days), but using a different data set (Engelbrecht *et al.,* 2008). Based on significant estimates of heterosis, crossbreeding between the SAB and ZB breeds seems to have a role to play in commercial production. However, further studies are required to modify this application for specific needs in the commercial industry. It needs to be ascertained whether similar benefits can be obtained from crosses between the SAB and the KR breeds.

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Chapter 4

Slaughter traits and physical meat quality of the South African Black, Zimbabwean Blue and the Kenyan Redneck ostriches as well as the crosses of the latter two lines with the SAB

Abstract

Three different ostrich breeds, namely the South African Black (SAB), Zimbabwean Blue (ZB) and the Kenyan Redneck (KR) and the cross between the SAB and ZB breed were assessed to determine the breed effects on physical meat quality traits. The traits measured were slaughter weight, dressing percentage, carcass weight, muscle pH24, cooking loss percentage, drip loss percentage, instrumental tenderness, and -colour. Slaughter weight of the SAB (86.5 kg) and ZB (93.9 kg) differed significantly, while the mean dressing percentage of the KR breed (52.5%) was improved relative to their SAB contemporaries (48.8%). The contrasts for carcass weight indicated that the ZB and KR breeds as well as the crosses were, on average, heavier than the SAB. Means for the muscle pH_{24} also differed ($P < 0.05$), with higher values for the ZB (6.36) and KR (6.4) relative to the SAB (5.85). Cooking loss percentage was found to differ significantly between the SAB (39.9%), ZB (35.6%) and KR (33.8%). No significant differences were observed between the breeds for drip loss % and instrumental tenderness. The instrumental b* colour value differed between the SAB (9.4) and KR (6.9). Crossbred performance resembled that of the purebred SAB birds for the meat quality traits (ultimate pH and b*-value). It was concluded that meat yield can be improved by utilizing the SAB breed as a dam-line with ZB and KR as sire lines in a commercial crossbreeding system, without compromising meat quality.

Introduction

Ostrich meat has become a global commodity (Hoffman, 2008) that is considered to be healthier than beef and other red meat types, because of its comparatively low intramuscular fat content (Sales, 1996). This has led to an increased global demand for ostrich meat. Successful ostrich production is crucial to supplying this increased global demand. Chick mortality rates are high and this is a major stumbling block for producers (Cloete *et al.*, 2001; Cloete *et al.*, 2002). Crossbreeding is known as a tool to improve fitness traits in populations, and thus enhance overall performance in livestock industries. It is being applied and evaluated in the ostrich research environment to improve reproduction and production traits in ostriches. Survivability of Zimbabwean Blue (ZB) x South African Black (SAB) hybrid chicks has been improved in comparison to purebred ZB chicks (Essa & Cloete, 2006). Heterosis has also been obtained for growth traits as well as for chick survival until 30 days of age, by crossing the ZB and SAB breeds (Engelbrecht *et al.*, 2008). Moreover, it is thought that there are breed differences between the SAB, ZB and Kenyan Redneck (KR) in live weight traits (Jarvis, 1998). It was furthermore demonstrated that the reproduction of SAB females is more advanced than that of ZB birds (Brand *et al.*, 2005; Cloete *et al.*, 2008). To our knowledge the reproductive performance of the KR has not been evaluated in scientific research, but results of a comparative study of the reproductive traits and body measurements of purebred SAB, ZB and KR will be presented in Chapter 5.

Limited information is available on the meat output and meat quality of ostrich breeds and their crosses (Hoffman, 2005; 2008). The ZB and SAB slaughter birds have been found to differ in their physical but not

sensory meat characteristics (Hoffman *et al.*, 2008). The SAB has been domesticated for a relatively long time and were farmed with since the late 1800's, being initially selected for their feathers (Deurden, 1913). This resulted in frequent handling of the birds during the harvesting of the feathers and the birds being indirectly selected for ease of handling by human handlers from the past. It can therefore be contemplated that this breed may be able to handle slaughter stress better than the other breeds, resulting in its meat having a lower ultimate pH (Hoffman *et al*., 2008). This phenomenon can be explained by the process of post-mortem glycolysis that occurs in the muscle. Energy is stored in the form of glycogen in muscle fibres. Post-mortem, glycogen is broken down anaerobically and its end-product is lactic acid. Lactic acid accumulates and leads to a reduction of the pH of the meat. In response to such an external stressor adrenaline is secreted from the adrenal medulla. This activates the breakdown of glycogen through an enzyme known as protein kinase. Depletion of the glycogen reserves occur when the animal has been under pre-slaughter stress. This leads to insufficient lactic acid being deposited in the muscle fibres post mortem resulting in a higher ultimate pH and a characteristic dark colour referred to as dry, firm and dark or DFD meat. The ZB and KR are known to have a more cantankerous behaviour than the SAB (personal observation; Hoffman *et al.,* 2008) and therefore may undergo higher levels of anxiety than the SAB at slaughter. It was postulated that this behaviour can lead to the meat of ZB slaughter birds having a higher ultimate pH compared to SAB contemporaries (Hoffman *et al.*, 2008).

It is postulated that breed differences between the SAB, ZB and KR, can be combined to produce offspring that has a heavier slaughter weight at the commercial slaughter age of approximately 14 months but still having complimentary physical meat quality traits. Against this background, the effect of crossbreeding ZB and KR birds with the SAB on the quantitative and qualitative meat traits was investigated.

Materials and methods

Animals and management

This study was conducted using the ostrich genetic resource population at the Oudtshoorn Research Farm in the Western Cape Province of South Africa. The SAB and ZB breeds on the farm (Cloete *et al.*, 2008) have recently been complemented with 12 KR males and six KR females. The KR females were used for pure breeding, while the excess males were mated to SAB females. Breeds were allocated to two potential roles in a structured crossbreeding system for linear contrasts that assisted in the appraisal of outcomes from the study. From this perspective the SAB dam was foreseen to play the role of a dam-line, because of the proven better reproduction ability of this breed compared to the ZB (Cloete *et al*., 2008). In contrast the ZB and KR birds were foreseen to play the role of sire-lines in a structured crossbreeding system because they had a heavier live weight than the SAB (Jarvis, 1998; Engelbrecht *et al*., 2008; Hoffman *et al*., 2008), but a poorer reproduction rate (Cloete *et al.,* 2008).

Slaughter ostriches represented the following breeds (number in brackets) and crossbred combinations, namely the SAB (34 males and 30 females), ZB (seven males and six females), ZB x SAB (seven males and five females), KR (one male and four females) and KR x SAB (nine males and two females). These birds

were hatched during 2007 and 2008, and grown out together in different groups under the same environmental conditions. These birds all received a grower diet (10.5 MJ ME/kg and 160g protein/kg dry matter) *ad libitum*. Birds were slaughtered during October 2009 (45 males and 31 females) and in March 2010 (13 males and 16 females) at an average slaughter age of 437 ± 4.4 days. Birds weighing less 60 kg were not included in this study.

Pre- slaughtering process

The birds were transported from the Oudtshoorn Research Fa to the Oudtshoorn abattoir in a truck 24 hours before slaughtering. Birds were kept in lairage overnight (approximately 12 hours) in different holding pens and only water was available for consumption. Transport and lairage were according to the norms and standards as used in the industry (South African Ostrich Business Chamber, 2009).

Slaughtering process

The birds were electrically stunned (105- 110V current and 400-800mA for 10 seconds), where after both legs were shackled and the birds were hoisted onto an overhead dressing rail. Exsanguination occurred within 60 seconds of stunning by means of a complete ventral cut of the neck, just below the head, which severed both the carotid artery and the jugular vein on both sides of the neck. This was followed by thoracic sticking with a sharp knife to severe the major blood vessels, including those from which the carotid arteries arise. These birds were allowed to bleed for ten minutes, where after the feathers were harvested post mortem. The birds were than weighed and dressed using standard procedures (South African Ostrich Business Chamber, 2009). The dressing percentage is calculated as the carcass weight/slaughter weight*100. Carcass weight can be described as the two thighs hanging distal to the epiphysis of the tibia (Sales, 1999), with the neck and leg bones sawed no longer than 15 cm in length, with the rib cage (the fat pad is removed), wings and tail still attached (World Ostrich Association, 2008). The carcass was stored in a cooling room at the Oudtshoorn Abattoir for approximately 24 hours, and one thigh was collected the next morning to remove the sample (*M. iliofibularis*) used for the physical measurements of cooking loss percentage, drip loss percentage, instrumental tenderness and colour measurements.

pH

The pH of the *M. iliofibularis* was recorded 45 to 60 minutes after slaughter (pH_0), and then 24 hours (pH_{24}) post mortem by using a penetrating glass electrode of a Testo portable pH meter (Testo 205, AG Germany). The pH meter was rinsed in distilled water after every reading and recalibrated after every 5^{th} reading.

Cooking loss

The fan fillet (*M. iliofibularis)* of each bird was removed 24 hours post mortem, and two 1.5 cm thick meat samples were cut cross sectional to the muscle from each experimental bird and used to determine cooking loss and drip loss, tenderness and colour. Samples were weighed and placed into thin-walled plastic bags, which were placed in a water bath at 80°C for 1 hour. The bag with the sample was then removed from the water bath, placed in cold water to cool down, where after it was blotted dry on tissue paper and then

reweighed. Cooking loss was calculated as the difference in weight of the meat sample before and after cooking, expressed as a percentage of the former weight (Honikel, 1998).

Drip loss

Samples were weighed and then a cord was pulled through the apical end of each meat sample. Each meat sample was placed into an individual plastic bag, which was then inflated. It was ensured that the meat sample did not come into contact with the plastic surface, but remained suspended in the middle of the bag. The samples were then stored for 24 hours at 4°C. After 24 hours the samples were removed and blotted dry on tissue paper and reweighed. The difference of the weight before and after the procedure was quantified as the drip loss of the meat sample after being expressed as a percentage relative to the original weight (Honikel, 1998).

Toughness

Meat samples were cooked at 80°C for one hour. Five cylindrical cores were then cut from each sample with the grain using a 1.27 cm diameter bore. Shear-force was determined as the amount of force necessary to shear one 1.27 cm core of meat sample perpendicular to the grain. The Warner-Bratzler device was attached to an Instron 4444, and the shear force measured in Newton/1.27 cm. The sample was sheared at a cross head speed of 200mm/min.

Colour measurements

The colour of the meat was measured with a Colour-guide 45°/0° colorimeter (BYK-Gardner, USA. Cat no: 6692) after allowing a blooming period of 30 minutes. Three measurements were taken on different parts of the meat sample and the mean of the three readings were used in the statistical analysis. The lightness of the meat (L*-value), as well as the positions on the red-green range (a*-value), and the blue-yellow range (b*-value), were measured. These values were used to compute the hue angle and chroma value which is indicative of the colour intensity and colour saturation respectively. The following formulas were used to determine the respective values:

Formula 1: Hue angle: $h_{ab} = \tan^{-1}\left(\frac{b*}{a*}\right)$

Formula 2: Chroma: $C* = \sqrt{(a*)^2 + (b*)^2}$

Statistical analysis

A final dataset of 105 records were analyzed. The objective of the study was to determine if the breed of the individual bird has a significant influence on its slaughter traits. The traits analyzed were slaughter weight (SLW), carcass weight (CW), dressing percentage (DP), fan fillet weight (FFW - expressed as kg and also relative to carcass weight as a percentage), (*M. iliofibularis*), pH_0, pH_{24}, cooking loss % (CL), drip loss % (DL

%), tenderness, colour intensity (CI) and colour saturation (CS). Slaughter age was fitted as a covariate in the model to correct the quantitative traits (SLW, CW, DP and FFW) for the irregular slaughter ages of the birds. As slaughter age is used as a covariate in the statistical analysis, it was therefore deemed unnecessary to include slaughter date/batch in any statistical analyses. Data were found to be normally distributed and the overall means and standard errors for each trait were calculated using the General Linear Model (GLM) procedure of SAS (SAS, 2006). The proposed model was:

$Y_{ijklm} = \mu + S_i + G_j + A_k y + \epsilon_{ijklm}$

Where:

Y_{ijklm} = The 1st observation of the i^{th} breed with the k^{th} gender
μ = overall means
S_i = The fixed effect of the i^{th} breed
G_j = The fixed effect of the j^{th} gender
$A_k Y$ = slaughter age as covariate
ϵ_{ijklm} = residual variance

Tukey comparisons were applied to all the variables of the dataset to compute significant differences ($P < 0.05$) between treatment means. The respective regression coefficients and their standard errors were estimated. The linear regression model fitted was:

$$Y = \alpha + \beta X + \varepsilon$$

Where:

- α = intercept (value of Y, given that X = 0),
- β = the gradient
- ε = error, where we assume that $\varepsilon \sim N(0, \sigma_e^2)$

Linear contrasts were only computed for traits that showed significant differences between the means. These contrasts were to determine:
1) Differences between the designated purebred SAB and the purebred ZB and KR breeds
2) Differences between the designated purebred SAB and the crosses (ZB x SAB and KR x SAB)
3) Differences between the designated purebred ZB and -KR breeds and their crosses (ZB x SAB and KR x SAB)

Direct heterosis (H_D) was estimated using the mean mid-parent value from the three purebreds and the mean value of the two crosses.

Formula 3: $$H_D = \frac{(ZBxSAB + KRxSAB) - (SAB + ((ZB + KR) * 0.5))}{(SAB + ((ZB + KR) * 0.5))}$$

These H_D values were calculated using Microsoft Excel, 2003®. It is conceded that reciprocal crosses of dam line sires with sire line dams are needed for the accurate estimation of true H_D. The derived values can thus only be considered as a guideline pertaining to the direction and magnitude of H_D in the ostrich breeds studied, and further studies are needed for accurate estimates.

Pearson product-moment correlation coefficients between the physical meat quality traits (pH_0, pH_{24}, L-value, a*-value, b*-value, hue angle, chroma value, CL%, DL% and shear force) were computed using the CORR procedure in SAS (SAS, 2006).

Results and discussion

The interaction of gender and breed were excluded from most of the final analysis on the respective traits, as no significant interaction occurred between gender and breed. Gender also had no influence on most of the dependent variables, except for cooking loss%, L*-value as well as pH_0 where it was included in the model. The absence of a gender effect was not entirely unexpected; as data reviewed by Cloete *et al.* (2002) and also published by Meyer *et al.* (2003) reported an absence of sexual dimorphism in almost all ostrich production traits up to slaughter age. Similarly, as no interactions were noted, only the main effect of breed will be discussed and where appropriate the influence of age. Slaughter age significantly influenced SLW and DP. No interaction occurred between the breed of the individual and slaughter age, therefore a linear regression (the effect of slaughter age as the independent variable on SLW and DP as dependent variables) was fitted to the pooled data of all the breeds.

The F-value for the effect of breed was significant ($P < 0.05$) in the analysis on slaughter weight (Table 4.1). However, slaughter age had no influence on the carcass weight ($P > 0.05$). A significant difference was observed between the SAB (86.5 kg) *vs.* the ZB (93.9 kg) breeds. Slaughter weight of the ZB breed, expressed relative to the mean of the SAB breed, amounted to a difference of approximately 9%. This result is supported by Engelbrecht *et al.* (2008), who reported a difference of almost 10% in favour of the ZB breed relative to the SAB in 14-months live weight. The KR breed is reported to have a faster growth rate than the SAB and ZB reaching a weight of 125 kg at ten months of age (Jarvis, 1998; Shanaway & Dingle 1999) and 135 kg at maturity (Jarvis, 1998). The anticipated difference in slaughter weight between the KR and SAB failed to materialize in this study, absolute means for the former breed being intermediate between the ZB and SAB, and not different ($P > 0.10$) from either. However, the small sample size of the KR ($n = 5$) may have caused a bias in the data and this aspect warrants further research with larger sample sizes for comparison purposes. The regression of slaughter weight on slaughter age (measured in days) is: $a = 70.4 \pm 8.56$ and $b = 0.04 \pm 0.02$. This indicates that at the slaughter age of approximately 14 months (427 days) the expected slaughter weight is 87.5 kg across breeds. Carcass weight means differed ($P < 0.05$) between the SAB and ZB. Linear contrasts indicated that the crosses (ZB x SAB and KR x SAB) were heavier ($P < 0.05$) than the purebred SAB birds (designated as a potential dam line in a structured crossbreeding system). The carcass weights of the purebred ZB and KR (designated as potential sire-lines) were similar to those of the

crosses. The designated sire-lines (purebred ZB and -KR) were found to be heavier than the designated dam-line (purebred SAB) ($P < 0.05$) for carcass weight. These results seem to support an argument that a breed such as the ZB and the KR could be used as crossbred sire-lines to augment the growth performance of crossbred progeny produced with the SAB as a dam-line. The KR breed was observed to have a smaller slaughter weight (89.0 kg) than the ZB (93.9 kg) in absolute terms, but attained similar values for carcass weight and dressing percentage. This phenomenon might be due to the differences in the amount of belly fat (fat pad) that is yielded by each breed, as carcass weight is mainly muscle weight excluding all the excess belly and subcutaneous fat.

Dressing percentage (DP) was significantly influenced by breed and also by slaughter age. The mean dressing percentage of SAB (49.8 ± 0.32 %) differed ($P < 0.05$) from that of the KR (52.5 ± 1.40 %), as well as the KR x SAB cross (53.8 ± 1.09 %); whereas there was no other differences found between the DP means of the other combinations (Table 4.1). Recent literature reported no differences in DP between the SAB and ZB breeds (Hoffman *et al.,* 2007). Linear contrasts indicated that the DP of the SAB (as the designated dam-line) differed from mean DP of the ZB and KR which was designated as sire-lines ($P < 0.05$), as well as from the mean DP of the crossbreds ($P < 0.05$). The mean DP of the purebred ZB and –KR birds (designated as sire-lines) was similar to the mean DP for the crosses. The coefficients for the regression of dressing percentage on slaughter age are: $a = 59.2 \pm 2.73$ and $b = -0.02 \pm 0.01$. The negative b-parameter (gradient) can be interpreted as a reduction in dressing percentage with an increase in slaughter age. This may be due to an increase in fat deposition in the belly of the ostrich, as the bird matures, and this fat is not included in the carcass weight (Hoffman *et al.,* 2005). This indicates that at a slaughter age of 14 months (427 days) the expected dressing percentage will be 50.7%.

Slaughter age had no effect on the weight of the *M.iliofibularis* expressed in kg or as a percentage against carcass weight. The weight of the *M.iliofibularis* tended to differ between the SAB breed and the ZB x SAB breed ($P = 0.06$; Table 4.1). The overall means for the SAB differed from both the crosses and the KR and ZB ($P < 0.05$). The means for designated sire-lines (ZB and KR) did not differ from those of the crosses ($P > 0.05$). A higher weight for the *M.iliofibularis* is preferable as it is one of the muscles that yield the highest income (Mellet, 1992).

Table 4.1 Means (± SE) depicting the effect of breed on slaughter weight, carcass weight, dressing percentage and fan fillet weight.

BREED	SLAUGHTER WEIGHT (KG) (CV*=10%)	CARCASS WEIGHT (KG) (CV=10%)	DRESSING PERCENTAGE (CV=5.1%)	FAN FILLET WEIGHT (KG) (CV=12%)
SAB (N=64)	86.5^{A}±1.0	43.1^{B}±0.5	49.8^{B}±0.32	1.53^{B}±0.02
ZB (N=13)	93.9^{B}±4.0	48.3^{A}±1.8	52.0^{AB}±0.64	1.64^{AB}±0.08
ZB X SAB (N=12)	92.9^{AB}±2.1	47.9^{A}±1.0	51.7^{AB}±0.83	1.69^{AB}±0.04
KR (N=5)	89.0^{AB}±3.0	46.9^{AB}±1.6	52.5^{A}±1.40	1.70^{A}±0.09
KR X SAB (N=11)	86.8^{AB}±2.2	46.6^{AB}±1.1	53.8^{A}±1.09	1.67^{AB}±0.04

[a-b] Column means with common superscripts do not differ (P > 0.05)
*Coefficient of variation

There were significant differences between breed means for pH_0 as well as for pH_{24}; with the SAB breed differing significantly from the ZB and the KR (Table 4.2). The means for pH for the crossbred breeds (ZB x SAB and KR x SAB) were intermediate in both instances, with the former breed resembling the SAB more closely. The higher pH found in the ZB and KR might be due to a more nervous behaviour prior to slaughter and resulting in increased stress levels (personal observation; Hoffman *et al.,* 2008). This behaviour causes rapid depletion of their glycogen reserves ante-mortem and therefore a small amount of lactic acid as product from the post mortem anaerobic glycolysis is available in the meat, resulting in a high ultimate pH. The means for the KR x SAB cross and the purebred ZB did not differ (P > 0.10). The contrast between the value of pH_0 and pH_{24} of the designated dam-line (SAB) differed from that of the designated sire-lines (ZB and KR, P < 0.05), while it was generally similar to the overall mean of the crosses. The mean pH_0 and pH_{24} of the designated sire-lines (ZB and KR) differed from that of the crosses (P < 0.05). A pH between 5.8 and 6.2 is considered as intermediate meat type, while pH above 6.2 can result in DFD meat. The lower pH of the SAB and the crosses is more favourable than that of the ZB and KR breeds, as the meat from the latter tends to be DFD and would have all the negative quality traits associated with this phenomenon (Lawrie & Ledward, 2006). These results indicate that the unfavourable pH of the designated sire-lines (ZB and KR) can be improved in crossbred progeny produced with the SAB as a dam-line. These results concur with those of Hoffman *et al*. (2008). In the case of purebred ZB birds, the confirmation of results by Hoffman et al. (2008) is reassuring, as it was conceded that the number of ZB observations in the previous study was very small (n = 2).

Drip loss % was not influenced by the breed of the bird (Table 4.2). These results did not agree with those of Brand (2006) who reported differences in drip loss % for the *M. iliofib* between the SAB (2.1%) and ZB (0.7%). Higher drip loss % was observed in the present study for the SAB (2.3%), ZB (2.4%) and for the SAB x ZB (2.4%). The difference from the study by Brand (2006) may be the result of coincidence, as only two ZB birds were used in the latter study, whereas 13 ZB experimental units were available for the present study. The magnitude of drip loss (2.2%) was consistent with that found by Joubert (2003) in the SAB breed.

Table 4.2 Means (± SE) depicting the meat quality traits of the SAB, ZB, KR breeds and their respective crosses.

Breed	N	pH_0	pH_{24}	Drip loss %	Cooking loss %	Toughness (N*)
SAB	64	5.73±0.05	5.85^{d}±0.04	2.3±0.11	39.9^{a}±0.4	40.4±0.7
ZB	13	6.20±0.12	6.36^{ab}±0.10	2.4±0.23	35.6^{bc}±1.2	41.7±2.3
ZB x SAB	12	5.95±0.09	6.06^{dc}±0.09	2.4±0.32	38.5^{a}±0.7	42.8±3.3
KR	5	6.40±0.13	6.40^{a}±0.12	3.3±0.59	33.8^{c}±2.5	45.1±4.0
KR x SAB	11	6.04±0.07	6.14^{bc}±0.07	3.1±0.45	37.9^{ab}±0.7	43.9±3.7

[a-d] Column means with common superscripts do not differ (P > 0.05)
N = Newton

Significant differences were obtained for the means of the breeds for cooking loss percentage. CL% were found to be highly negatively correlated to pH24 (r = -0.80) for the pooled data of all the breeds (Table 4.4). This negative correlation is verified by some breeds (SAB and the crosses) that exhibit a low pH value correspondingly having a high CL% or other breeds (ZB and KR) that exhibit a high pH and correspondingly have a lower CL% (Table 4.2). As observed, CL% is the highest for the SAB breed (39.9%) and the lowest for the KR (33.8%; Table 4.2) (P < 0.05). This ranking corresponds with their pH_{24} values where the SAB had a lower pH_{24} and a reduced water holding capacity (WHC), with the reverse being true for the KR. A high WHC may be detrimental as it allows for shorter shelf-life as high-pH meat becomes more susceptible to microbial spoilage (Lawrie & Ledward, 2006). Microbes flourish in areas that have a pH close to 7 and with high moisture content. Designated contrasts between breed combinations indicated that the cooking loss of the SAB did not differ from those of the crosses (P > 0.05). However, the means of both the dam-line (SAB) as well as crosses differed from those of the sire-lines (ZB and KR) (P < 0.05) for cooking loss. Instrumental toughness was independent of all the effects included in the fitted model. These results were consistent with those of Hoffman *et al*. (2008) on the SAB, ZB and the ZB x SAB cross. The latter study also found no apparent breed differences in sensory meat tenderness.

Cooking loss % was also found to be positively correlated with most of the meat colour traits (Table 4.4). Therefore an increase in CL% is analogous to an increase in L*-value, a*-value, b*value, hue angle and chroma value, as observed in Table 4.2 and 4.3.

As pertaining to the meat colour ordinates the SAB did not differ (P > 0.05) from the ZB, ZB x SAB and KR x SAB crosses however, there was a difference (P < 0.05) between the b*-values of the SAB (9.38) and KR (6.87; Table 4.3). The SAB meat was thus situated more towards yellow on the blue-yellow axis than KR meat. The chroma means for the SAB (19.1) and KR (15.8) also tended to differ, with the P-value approaching significance (P = 0.06). A higher chroma value indicates more colour saturation, thus the SAB meat appears brighter, and more saturated with colour compared to KR meat (P = 0.06). Meat from the KR breed appears dark, but has a lower level of colour saturation which is typical colour characteristics of DFD

meat. These results might be because the KR breed is more nervous (e.g. typically running around more than the SAB birds in the lairage pen in order to prevent being captured by the handlers) than the SAB ante mortem (personal observation) which therefore causes most of its glycogen reserves to be depleted, resulting in a high ultimate pH. The two crosses resembles the designated dam-line (SAB) in reference to pH_{24}, whereas they performed intermediate between the dam and sire-lines (SAB and ZB and KR breeds; midparent value) as pertaining to the b*-value and chroma value. The pH value of meat is known to be negatively correlated to the colour of meat (Lawrie & Ledward, 2006) as depicted in Table 4.4. A low ultimate pH leads to meat appearing brighter (L*-value: r = -0.23, chroma value: r = -0.58) and more red than meat that reaches a higher ultimate pH (a*-value: r = -0.52 and hue angle: r = -0.53). This trend of negative correlation between pH and colour traits can be typically seen represented in all the breeds (Table 4.2 and Table 4.3).

Table 4.3 Means (± SE) depicting the meat colour traits of the SAB, ZB, KR and their respective crosses.

Breed	N	L*-value	a*-value	b*-value	Hue angle	Chroma value
SAB	11	30.7±0.3	16.6±0.2	9.38^{a}± 0.2	29.3±0.4	19.1^{a}± 0.3
ZB	13	28.3±1.2	15.4±0.7	8.07^{ab}±0.5	27.5±1.0	17.4^{ab}±0.8
ZB x SAB	12	30.8±1.1	15.7±0.7	8.13^{ab}±0.6	26.9±1.1	17.7^{ab}±0.9
KR	5	29.7±1.7	14.2±0.9	6.87^{b}± 0.7	25.5±1.4	15.8^{b}± 1.1
KR x SAB	64	32.6±2.0	15.5±0.6	8.40^{ab}±0.6	28.3±1.0	17.7^{ab}±0.8

$^{a-b}$ Column means with common superscripts do not differ (P > 0.05)

The colour traits are all positively correlated to each other, except for the L-value, as observed in Table 4.4. The a*-value is positively correlated to the b*-value, hue angle and chroma value, whereas the b*-value is positively correlated to hue angle (colour intensity) and chroma value (colour saturation). These abovementioned traits are all expected to be interrelated since the a*- and b*-values are both components that contribute to colour intensity and colour saturation (Formula 1 and 2).

No significant differences were observed between the tenderness of the five breeds although their pH values differed as it is known that muscle pH influences proteolytic enzyme activity and thus tenderness (Purchas, 1990). A graph was therefore plotted with the pooled data of all the breeds to relate ultimate pH to tenderness (Figure 4.1). The relationship between pH and tenderness is known to be curvilinear - with low and high pH values having more tender meat (Purchas, 1990). However, no regression equation fitted gave an accurate estimation of this relationship.

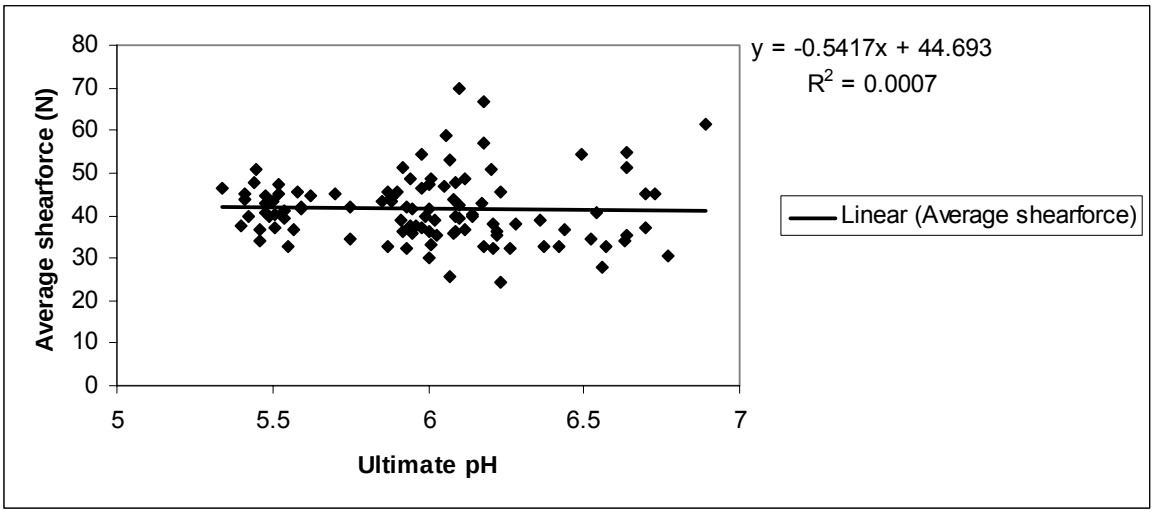

Figure 4.1 The influence of ultimate pH on the tenderness (N) of meat for the pooled data of all the breeds.

Table 4.4 Phenotypic correlations among physical meat traits of pooled data of all the ostrich breeds.

	pH_{24}	L	a	b	Hue angle	Chroma	CL %	DL%	Shear force (N)
pH_0	0.94*	-0.28*	-0.50*	-0.63*	-0.56*	-0.57*	-0.83*	0.28*	-0.05
pH_{24}		-0.23*	-0.52*	-0.62*	-0.53*	-0.58*	-0.80*	0.36*	-0.03
L			-0.29*	-0.01	0.19	-0.20*	0.43*	0.36*	0.04
a				0.84*	0.49*	0.98*	0.47*	-0.32*	0.03
b					0.87*	0.93*	0.60*	-0.27*	0.11
Hue angle						0.65*	0.56*	-0.19*	0.17
Chroma							0.53*	-0.31*	0.06
CL%								-0.05*	0.08
DL%									-0.16

*Depicting significant correlation values ($P < 0.05$)

CL% = cooking loss %

DL% = drip loss %

Values of direct heterosis were generally low (< 6%). Traits where direct heterosis exceeded 2% were carcass weight (4.2%), dressing percentage (3.4%), fan fillet weight (5%) and cooking loss (2.4%). The only previous heterosis estimate for similar traits in ostriches was a level of 6.7% heterosis that had been estimated for 14-months-weight (which could serve as a proxy for slaughter weight) in ostriches (Engelbrecht *et al.*, 2008). This estimate is substantially higher than an estimate of 1% derived from all breed combinations in the present study. The estimate published by Engelbrecht *et al*. (2008) sourced data from SAB and ZB ostriches, as well as their reciprocal cross (n = 610). When only SAB and ZB data were sourced from the present study, a heterosis estimate of 3% was derived, which would be closer to the report by Engelbrecht *et al*. (2008). Heterosis estimates for meat pH and colour were all below 2% ($P > 0.50$).

Table 4.5 Heterosis estimates for carcass traits in ostriches.

Trait	Estimated heterosis	P-value
Carcass weight	4.2%	0.11
Slaughter weight	1.0%	0.69
Dressing %	3.4%	0.01
Fan-fillet weight	5.0%	0.11
Cooking-loss %	2.4%	0.44
pH (0)	-1.88%	0.74
pH24	-1.66%	0.79
b*-value	1.95%	0.73
Chroma-value	1.52%	0.81

Conclusion

There is strong evidence that ZB birds outperformed their SAB contemporaries for live weight at slaughter, as had been reported in previous studies. In contrast, these first indications of the performance of the KR breed for live weight were less satisfactory, and further studies are indicated. However, a better dressing percentage in the latter breed resulted in it being fairly competitive as far as carcass weight was concerned. On the other hand, meat quality traits such as pH and meat colour were superior in the SAB breed. Crossbred performance was generally supportive of a contention that sustainable crossbreeding options are available for ostriches.

In attempting to estimate heterosis, the present study had a number of limitations, namely 1) reciprocal crosses of the SAB breed with the ZB and KR breeds were not available; 2) the number of animals representing some genetic groups were less than optimal, increasing the probability of coincidence; 3) animals achieving superior mature live weight from the ZB and KR breeds and their crosses were retained for a subsequent study on heterosis of reproduction traits. Although obvious runts were not considered in the slaughter study, it still leaves the possibility that mediocre animals were overly represented in those animals of these breeds that were slaughtered.

The SAB breed has been confirmed to exhibit proficient egg and chick production (chapter 5) and meat quality traits. The ZB and KR exhibit excellent growth performance (chapter 3) and carcass traits. Therefore it would be viable to use the SAB breed as a dam-line and the ZB and KR as sire lines in a structured crossbreeding system to exhibit favourable meat quantitative and qualitative traits.

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Chapter 5

Breed effects on live weight, body measurements and reproduction of South African Black, Zimbabwean Blue and Kenyan Redneck ostriches, as well as maternal heterosis in crosses between the former two lines

Abstract

Live weight, body measurements and reproduction of South African Black (SAB), Zimbabwean Blue (ZB) and Kenyan Redneck (KR) ostriches were investigated to determine differences between these breeds. A reciprocal crossbred design was also applied to the SAB and ZB breeds. Traits analysed were live weight, front chest circumferences, tail circumference, total egg production, the number of fertile eggs, infertile eggs, number dead in shell chicks, hatchability and chick production as well as the time to lay. The ZB and KR were heavier in live weight and of larger body measurements than the SAB, whereas the SAB exhibited superior reproduction performance in comparison to the ZB and KR breeds. From the SAB and ZB cross, the crossbred progeny resembled the ZB breed in the performance of live weight and body measurements, whereas the crossbred females resembled the SAB breed in terms of reproduction performance. Maternal heterosis was also estimated from the mid-parent value (MPV) for all the above mentioned traits. Maternal heterosis estimated for tail circumference was 2.2%, 12% for total egg production, 12% for hatchability and 19% for chick production. It thus seems as if crossbred females have a role to play in commercial production, as with other livestock species.

Introduction

Viable day-old ostrich chicks are a commodity with economic value in the South African industry. Yet chick production of domesticated ostriches is low and variable (Cloete *et al.*, 2008). Hatching success is often constrained by high levels of shell deaths (Brand *et al.*, 2008), as well as low fertility of artificially incubated ostrich eggs (Bunter, 2002).

The genetic resource available to the South African ostrich industry includes the South African Black (SAB), Zimbabwean Blue (ZB) and Kenyan Redneck (KR) breeds. The reproduction of these breeds have not been adequately quantified as of yet. Although Cloete *et al.* (2008) reported the mean mature live weights and reproduction of the SAB and ZB breeds; it was conceded that the ZB birds included in the study was a largely unquantified resource. Limited information on the origin of the birds was available, except that some of them originated from the Harare region, while others originated from Bulawayo. No information on age was available, while it is known that age affects reproduction in the SAB breed (Bunter, 2002; Cloete *et al.,* 2006). Further studies on the live weight and reproduction of the ZB birds with a known ancestry and age structure are thus needed to confirm or refute the earlier conclusion made by Cloete *et al.* (2008). The SAB were found to be superior to the ZB in reproduction (Cloete *et al.*, 2008), and the ZB (Jarvis, 1998; Cloete *et al.*, 2008) as well as the KR (Jarvis, 1998) are expected to be superior in live weight. Furthermore, the KR remains an unquantified resource which needs to be assessed for mature live weight and reproduction.

Crossbreeding is a known breeding strategy to improve fitness, reproduction and other lowly heritable traits in livestock. Crossbreeding can be defined as the intentional mating of individuals originating from different populations, breeds or breeds (Nicholas, 1996). The use of crossbred progeny allows the exploitation of non-additive genetic variation known as heterosis. Heterosis is attained when the average performance of reciprocal crossbred progeny is superior to the average performance of the two parental breeds (Nicholas, 1996). It can be divided into individual heterosis, paternal heterosis and maternal heterosis. Maternal heterosis is the non-additive genetic constituent of the dam that influences the oviductal environment for egg development in avian species. Maternal heterosis could influence egg and chick production and thus improve the reproductive performance of the dam. Maternal heterosis on reproductive traits has not been studied in ostriches.

Therefore the aim of this study was to investigate breed or breed effects, as well as the effect of crossbreeding between the SAB and ZB breeds on reproduction traits of ostrich females.

Material and Methods

Experimental animals

Reproductively mature ostrich breeding birds of the Oudtshoorn Research Farm were used for this study. This breeding flock was developed through the donation of 76 South African Black (SAB) by 61 local producers in 1964. Later introduction of feather birds followed in the 1990's, while other commercial birds of industry origin were introduced in 2002. The breeding flock has increased in size from the original 33 breeding pairs (Van Schalkwyk *et al.,* 1996), and currently consists out of 188 breeding pairs. In 2003, 55 Zimbabwean Blue (ZB) ostriches were added to the flock, as obtained from two local producers. These birds were originally from the Bulawayo and Harare areas of Zimbabwe (Engelbrecht *et al.*, 2008). In 2004, 19 Kenyan redneck (KR) ostriches, of which 13 were males and six females, were introduced to the flock.

Birds used for this study were bred in pairs instead of colonies to ensure that complete pedigree records were available for their progeny. The breeding season initially commenced during late-May and ceased at the end of January (2005 to 2007) and were shifted to commence during mid-May until cessation in mid-December (2008 to 2009). Birds were fed 2.5 kg breeder diet per day during 2005 to 2006 and 3 kg per day from 2007 to 2009. The birds had received water ad libitum throughout the breeding season. A resting period from the cessation of breeding until the commencement of the next years breeding was allowed each year and the pairs were divided into male- and female-specific flocks during this period. During the resting period the birds were fed maintenance diets based on hammer-milled Lucerne (*Medicago sativa*) with an energy content of 9.1MJ ME/kg and 133 g protein/kg dry matter. These resting birds were also allowed to graze on lucerne pastures. Birds were spray-dipped with Bayticol (of which the active component is flumethrin) to protect them against external parasites. They were vaccinated against Newcastle disease, necrotic enteritis and influenza. The breeding pairs were flushed with a high energy diet containing 10MJ ME/kg and 160g protein/kg dry matter from two weeks before the beginning of the breeding season. At the commencement of breeding season, females and males constituting pairs were released into 0.25 ha breeding paddocks containing shrubs, naturally occurring in the Klein Karoo area. All females were weighed and measured for

front chest circumference as well as tail circumference as described by Cloete *et al.* (2006) at the commencement and cessation of breeding during each breeding season.

Egg collection

The collection of the eggs occurred every morning and during the late afternoon throughout the breeding season. The eggs were identified by the paddock number and date of collection as described by Bunter (2002). Data of the eggs weighing less than 1.2 kg were not included in the trial. Eggs were uncontaminated by using a tissue paper to remove exterior dirt and than was transferred into an UV disinfectant machine for 20 minutes (Prohatch®) for further sanitation. After sanitation the eggs were conveyed to a cool room with a temperature of 17 °C and 75% relative humidity. The air cell of each egg was determined by shining a torch on the egg while in the dark cool room. The position of the air cell was marked and the eggs were stored with the air cell facing upwards in the cool room for a maximum of six days for the majority of eggs. Longer storage periods were only applicable for those eggs that were collected at the beginning and end of breeding seasons, when the weekly number of eggs collected did not validate the setting of a separate batch. The eggs were removed from the cool room and placed in one of the following incubators; Buckeye®, Prohatch® and Natureform® and African International ® electronic incubators for 38 days at 36% and a relative humidity of 24%. Fogging of eggs occurred weekly with F-10 for sanitation. The process involving the handling and incubation of eggs were also described by Brand *et al.* (2008). Candling occurred on day 21 to see if there are any early embryonic deaths, and also for fertility and on day 35 to check for late embryonic deaths. After 35 days of incubation, the eggs were removed to the hatching unit, which operated at 36% and 28% relative humidity. The eggs were held vertically within the hatcher unit and no turning occurred until external pipping took place at approximately 42 days. The chicks were kept in separate compartments to facilitate identification, and kept in the hatcher for 24 hours to allow time for their navels to close and for the chicks to dry (Brand *et al.,* 2008).

Experimental design

Reproductive data as well as live weight and body dimensions was recorded for the purebred SAB, ZB and KR dams from 2006 to 2010. These records were used to compare reproductive performance, live weight and body dimensions in the three purebred ostrich breeds namely the SAB, ZB and KR. The breed of the female was entered as a fixed effect in the statistical analysis described below.

For the crosses, the experimental design was a reciprocal crossbred design for the ZB and the SAB breeds. This design was suitable to estimate heterosis. No reciprocal cross was performed between the SAB and KR breeds because there were an insufficient number of KR females available on the farm. Therefore maternal heterosis (%) could only be reliably estimated for the SAB x SAB, ZB x ZB, SAB x ZB and ZB x SAB combinations. The reproductive data, live weight and body dimensions of F1 dams were recorded from 2005 to 2010.

<u>Reproductive traits measured were:</u>

- Total egg production

- Number of fertile eggs laid
- Number of dead in shell chicks
- Hatchability (the number of chicks produced over the number of fertile eggs produced)
- Number of chicks produced per breeding season
- Time to lay (defined as the interval between the commencement of mating and the production of the first egg).

Although mature weight was not expected to benefit from heterosis, information will also be supplied on live weight and body dimensions, namely front chest circumference and tail circumference.

Statistical Analysis

The general linear models (GLM) procedure of SAS (2006) was applied to the data to determine the effect of breed of the dam on the respective traits for purebred SAB, ZB and KR females. The independent variables were breed of the dam, female age and the year of breeding season. The interaction between female age and year was also fitted and the number of days in the breeding season was fitted as a linear covariate to egg production traits. Significant ($P < 0.05$) interactions were observed for year by female age. As these interactions did not affect breed means, they were not presented or discussed. Bonferonni comparisons were applied between the treatment means for breed of the dam for each trait. Model 1 notated below was run firstly to determine differences between the three breeds; SAB, ZB and KR for which 310 hen-year production records were available in total.

Model 1:

$y_{ijklm} = \mu + G_i + F_j + Y_k + (FY)_{jk} + D_l y + \epsilon_{ijklm}$

Where:

μ = The overall mean

y_{ijkl} = The m^{th} reproduction, live weight or body measurement record of the i^{th} breed with the j^{th} female age and recorded in the k^{th} year

G_i= The fixed effect of the i^{th} breed

F_j= The fixed effect of the j^{th} age of the female

Y_k= The fixed effect of the k^{th} production year

$(FY)_{jk}$ = The interaction of the j^{th} age of the female in the k^{th} year of breeding

$D_l y$= Length of the breeding season, fitted as a linear covariate

ϵ_{ijklm} = residual error

Secondly, the same basic model was applied to 420 hen-year records to determine differences between the performance of SAB, ZB, SAB x ZB and ZB x SAB females. Linear contrasts were estimated between the midparent value (MPV) and the mean crossbred performance to determine the effect of maternal heterosis for each trait recorded on the SAB and ZB females as well as the reciprocal crosses between these breeds. Maternal heterosis for each trait was calculated in Microsoft Excel ® 2003, using the following equation:

$\%H = P_{F1} – P_p / P_p X 100$ (Bourdon, 2000)

Where:

%H = Percentage heterosis

P_{F1} = mean of F1 crossing

P_p = mean of parental breeds

Results and Discussion

In general, it needs to be stated that results pertaining to female age and production years were consistent with results from literature; in that the reproduction of two year-old females was inferior to that of older females (Bunter *et al.*, 2001; Cloete *et al.*, 2006). The quantitative reproduction traits (egg and chick production) increased with an increased length of production season as was reported previously by Bunter *et al.* (2001) and Cloete *et al.* (2006). These results, as wells as significant interaction effects between female age and production year were thus excluded from further discussion. The reason for this was that these results did not contribute to the primary objective of the study, which was to study breed effects and maternal heterosis.

Performance of purebreds (SAB, ZB and KR)

Breed of the dam significantly influenced mean live weight both at the commencement and cessation of the breeding season ($P < 0.05$). However, breed of the dam had no influence on the live weight and front chest circumference, which is in accordance with the study done by Brand, (2006). The least square means for live weight and body measurements of the SAB and ZB are in agreement of those reported by Brand (2006). The ZB and KR breeds were generally heavier in live weight than the SAB breed.

At the commencement of the breeding season, the ZB breed was 13% heavier than their SAB contemporaries (Table 5.1). The ZB breed also outperformed the SAB for live weight at the cessation of breeding, ZB females being 14% higher in live weight ($P < 0.05$). These results are consistent with a report that the ZB females were between 10.4% and 12.6% heavier than SAB females (Cloete *et al.*, 2008).

Females of the KR breed were generally heavier in live weight than the SAB breed ($P < 0.05$). The weight advantage of the KR females amounted to 13% at the commencement of breeding and 14% at the cessation of breeding. These results confirm the fact that the KR is heavier in live weight when compared to the SAB breed (Jarvis, 1998). However no differences were observed between the KR and ZB for either live weight or the body measurements recorded. This result seems to contradict the suggestion of Jarvis (1998) that mature KR birds are about 10 kg heavier (135 vs. 125 kg) than ZB contemporaries. However, further research is necessary to accurately quantify the breed effects of the KR breed.

Table 5.1 Least square means (± SE) depicting the influence of breed (SAB, ZB or KR) on live weight and body measurements.

Traits	CV	Breed		
		SAB (n=268)	ZB (n=34)	KR (n=8)
Beginning of season				
Live weight (kg)	19.2%	113[b]±1.48	130[a]±2.21	130[a]±5.95
Front chest circumference (cm)	17.6%	118±1.39	125±0.84	126±1.90
Tail circumference (cm)	18.1%	115[b]±1.39	122[a]±0.84	121[a]±2.48
End of breeding season				
Live weight (kg)	15.0%	106[b]±1.03	123[a]±4.81	123[a]±6.13
Front chest circumference (cm)	11.2%	121±0.82	123±3.99	121±2.07
Tail circumference (cm)	11.6%	115[b]±0.94	122[a]±4.02	121[a]±3.45

[a-b] Row means with common superscripts do not differ (P > 0.05)

Coefficients of variation calculated in this study for the number of infertile eggs, egg production and chick production are mostly consistent with those reported in the literature (Bunter, 2002, Cloete *et al.,* 2005). Comparable coefficient of variation reported by Cloete *et al.* (2005) were 59% for total egg production, 52% for hatchability, 84% for chick production and 96% for time to lay. Corresponding values in the present study were 40%, 37%, 64% and 76%. Slightly lower coefficients of variation for some traits in the present study could be attributed to the fact that the present data was recorded in recent years (2005-2009), while the study of Cloete *et al.* (2005) spanned the period from 1991 to 2003. Recent records may possibly show less variation because of improvements in husbandry, as well as possible selective breeding that took place over the years.

The SAB performed superior to the ZB breed as far as reproduction was concerned (Table 5.2). Relative to the ZB breed, the SAB females produced, 63% more eggs, 57% more fertile eggs, maintained a 12% better hatchability, produced 69% more chicks and had a 34% shorter time to lay the first egg. In relation to the SAB breeds' total egg production 18% were infertile eggs, and 19% dead in shell eggs *vs.* the ZB who produced 12% infertile eggs and 18% dead in shell eggs in relation to total egg production (Table 5.2). The results for reproduction traits (total egg production, hatchability and chick production) found in this study support literature that reported the SAB breed to have an improved reproduction compared to the ZB breed (Brand *et al.*, 2005; Cloete *et al.*, 2008). However the magnitude of breed differences seemed to be higher in the study of Cloete *et al.* (2008). Key reproduction outputs (egg production and chick production) for the respective studies were thus reported in Table 5.3. Cloete *et al.* (2008) conceded that the fact that the ZB birds used in their study were not well quantified, as no information on ancestry or age were available. The conclusion of Cloete *et al.* (2008) pertaining to the superior reproduction of the SAB breed relative to ZB contemporaries is confirmed in the present study, although the magnitude of the difference between breeds is smaller. The difference between studies may be ascribed to the fact that the ZB resource in this study

were all hatched and reared on the Oudtshoorn Research Farm, thereby being similar for ancestry and age to their SAB contemporaries.

The SAB breed also outperformed the KR in terms of reproduction. Relative to the KR, the SAB produced 22% more eggs, 24% more fertile eggs, 28% less dead in shell eggs, maintained a 32% better hatchability, and produced 69% more chicks while its interval for time to lay was 12% lower than that of the KR breed. In reference to its total egg production the KR produced 18% infertile eggs which are similar to the percentage of infertile eggs produced by the SAB breed. From these results, it is evident that the SAB breed reproduced better than the KR females in the present study. Although some breed differences were found between the ZB and KR, the key reproduction measures (egg production and chick production) did not differ between these breeds. It is notable that the KR females were more likely to produce dead in shell eggs than their ZB contemporaries. No results could be sourced from literature to support or refute these findings on the comparison of the KR females with their SAB and ZB contemporaries.

Table 5.2 Least square means (± SE) depicting the influence of breed (SAB, ZB or KR) on reproduction traits.

Reproduction Traits	CV	Breed		
		SAB (n=268)	ZB (n=34)	KR (n=8)
Total egg production	40%	44^{a}±1.2	27^{b}±2.43	36^{ab}±5.4
Number of fertile eggs	49%	36^{a}±1.2	23^{b}±2.2	29^{ab} ± 5.6
Number of infertile eggs	117%	8.1^{a}±0.56	3.2^{b}±0.97	6.3^{a}±2.3
Number of dead in shell eggs	77%	8.7^{ab}±0.45	4.9^{b}±0.87	12^{a}±2.48
Hatchability	37%	0.58^{a}±0.01	0.52^{b}±0.04	0.44^{c}±0.07
Chick production	64%	22^{a}±0.86	13^{b}±1.4	13^{b}±3.7
Time to lay	76%	57^{b}±3.2	87^{a}±13	65^{b}±10

$^{a-c}$ Row means with common superscripts do not differ (P > 0.05)

Table 5.3 Least square means (± SE) for egg production and chick production of the breeds in analogous studies (SAB and ZB) for the present study *vs.* the previous study of Cloete *et al.* (2008).

Study	Total egg production		Chick production	
	SAB	ZB	SAB	ZB
Present study	44.0±1.2	27.0±2.4	22.0±0.86	13.0±1.4
Cloete *et al.* (2008)	43.3±2.1	23.3±3.6	23.1±1.5	10.6±2.5

The performance of crossbred females and the estimation of heterosis

Sufficient data were available to allow the estimation of heterosis in the reciprocal cross between the SAB and ZB breeds. Although the derived linear contrasts between the mean crossbred performance and the

midparent value were not significant in many instances, the derived mean values were still used to estimate the percentage of heterosis, as no comparable results are found in literature. The relative performance of the purebreds (SAB and ZB in this instance) reiterated the breed differences in Tables 5.1 and 5.2, namely that the ZB were heavier ($P < 0.05$) with a poorer reproduction ($P < 0.05$) in comparison with their SAB contemporaries. This is not surprising as the previous analysis also included the bulk of the records used in the present analysis. As these breed differences between the SAB and ZB were adequately covered in the previous section, the emphasis in this section will mainly be on the performance of the crossbreds.

The crosses were generally intermediate in magnitude between the pure breeds for live weight and body dimension traits (Table 5.4). At the commencement of breeding the ZB male and the SAB female combination were 8% lighter than the ZB purebred females ($P < 0.05$) but not different in live weight from their purebred SAB contemporaries. In contrast, the SAB male and the ZB female combination was 8% heavier than purebred SAB females ($P < 0.05$), but not different from their ZB contemporaries. At the cessation of breeding the ZB x SAB and the SAB x ZB combinations were respectively 9% and 11% heavier than the purebred SAB females ($P < 0.05$), but of similar live weight than their ZB contemporaries ($P > 0.05$). Breed effects were found to be insignificant for front chest circumference ($P > 0.05$). The mean value for tail circumference, of the ZB x SAB crossbreed was intermediate from the purebred SAB and ZB breeds and did not differ from either purebred breed at the commencement of breeding ($P < 0.05$), however tail circumference for the ZB x SAB differs ($P < 0.05$) from the SAB purebred at the cessation of the breeding season. In contrast, the mean value for tail circumference of the SAB x ZB crossbreed resembles that of the ZB purebred and differs significantly from the SAB purebred at the commencement and cessation of breeding season.

Table 5.4 Least square means (± SE) depicting the breed effect (SAB, ZB, ZB x SAB and SAB x ZB) on live weight and body measurements.

Traits	CV*%	Breed			
		SAB (n=268)	ZB (n=34)	ZB x SAB (n=94)	SAB x ZB (n=24)
Beginning of breeding					
Live weight (kg)	17%	113^{c}±1.48	130^{a}±2.21	120^{bc}±1.71	122^{ab}±2.32
Front chest circumference (cm)	14%	118±1.40	125±0.84	121±1.40	124±1.13
Tail circumference (cm)	15%	115^{b}±1.39	122^{a}±0.84	119^{ab}±1.46	123^{a}±1.39
End of breeding					
Live weight (kg)	15%	106^{b}±1.03	123^{a}±4.81	116^{a} ±1.93	118^{a}±2.89
Front chest circumference (cm)	11%	121±0.81	123±3.98	124±1.65	126±1.95
Tail circumference (cm)	14%	115^{b}±0.94	122^{a}±4.02	122^{a}±1.77	121^{a}±2.33

$^{a-c}$ Row means with common superscripts do not differ (P > 0.05)

*CV = Coefficient of variation

Heterosis estimated for live weight at the commencement of breeding season was -0.4%. No comparable results were found in the literature of ostriches, but this finding agrees with reports in the literature indicative of low maternal heterosis for mature live weight in beef cattle (Kress *et al.*, 1990). Maternal heterosis estimated for front chest circumference amounted to 0.8% at this stage, while maternal heterosis estimated for tail circumference was 2.1%. Estimated maternal heterosis for live weight at the cessation of breeding was 2.2% with the contrast of crossbred performance relative to the mid-parent value being insignificant (P = 0.34). Estimated maternal heterosis for front chest circumference at this stage was 2.2%, while maternal heterosis estimated for tail circumference amounted to 2.5 % (P = 0.11).

The low maternal heterosis for live weight and body measurements can be explained by the fact that heterosis is generally greater for lowly heritable reproduction traits than for growth traits (Fairfull, 1990). However higher percentages than observed in this study have been estimated for individual heterosis of live weight and body measurements in turkeys, chickens and beef cattle (Mc Elhenney *et al.*, 1985; Hanafi *et al.*, 1991; Nestor, 2001).

Table 5.5 Maternal heterosis estimated for live weight and body measurements as derived from SAB, ZB, ZB x SAB and SAB x ZB breeding birds.

Trait	Maternal Heterosis	P-value
Beginning of breeding		
Live weight	-0.4%	0.23
Front chest circumference	0.8%	0.50
Tail circumference	2.1%	0.04
End of breeding		
Live weight	2.2%	0.34
Front chest circumference	2.5%	0.71
Tail circumference	2.5%	0.11

In contrast to the results of live weight and body measurements, the crossbred females mostly resembled the SAB breed for reproduction performance. Relative to the ZB breed the ZB x SAB combination produced 32% more eggs, 26% more fertile eggs, 28% more chicks at an a 7% better hatchability, with a 28% shorter time to oviposition. Relative to the ZB, the SAB x ZB crossbred females produced 37% more eggs, 34% more fertile eggs, 43% more chicks with the hatchability improved by 17% and with a 28 % shorter interval to oviposition.

Table 5.6 Least squares means (± SE) depicting breeds effect of (SAB, ZB, ZB x SAB and SAB x ZB) females for reproduction traits.

Reproduction traits	CV	Breed			
		SAB (n=268)	ZB (n=34)	ZB x SAB (n=94)	SAB x ZB (n=24)
Total egg production	43%	44^{a}±1.2	26^{b}±2.4	38^{a}±1.9	41^{a}±3.1
Number of dead in shell eggs	79%	8.7^{a}±0.45	4.9^{b}±0.87	7.8^{a}±0.71	7.7^{a}±1.2
Number of fertile eggs	51%	36^{a}±1.2	23^{b}±2.2	31^{a}±1.8	35^{a}±3.1
Number of infertile eggs	121%	8.1^{a}±0.55	3.2^{b}±0.97	7.0^{a}±1.0	6.1^{a}±1.2
Hatchability	34%	0.58^{ab}±0.01	0.52^{b}±0.04	0.56^{ab}±0.02	0.63^{a}±0.04
Chick production	64%	22^{ab}±0.86	13^{c}±1.4	18^{b}±1.3	23^{a}±2.5
Time to lay	63%	57^{b}±3.2	87^{a}±13	68^{ab}±5.7	68^{ab}±9.2

$^{a-c}$ Column means with common superscripts do not differ (P > 0.05)

The linear contrast indicative of maternal heterosis was significant for hatchability and chick production (P < 0.05), and approached significance for total egg production (P < 0.07). It is therefore clear that these measures of reproductive performance will benefit from crossbreeding. Direct heterosis for egg production in chickens was observed to range between 2.7% and 5.5% depending on the crosses (Saadey *et al.*, 2008). Maternal heterosis for total egg production in ostriches as observed in this study approached significance

with an estimate of 12%. This agrees with an estimate of 12.3% that was reported for maternal heterosis of annual egg production in chickens (Khalil *et al.*, 2004). Day-old ostrich chicks are of commercial value in the ostrich industry, in contrast to poultry chicks. It is thus not surprising that no comparable poultry estimates were found to benchmark the present heterosis estimate of 19% for chick production against.

Table 5.7 Estimated Maternal Heterosis for reproduction traits in SAB, ZB, ZB x SAB and SAB x ZB females.

Traits	Estimated % H^m	P-value
Total egg production	12%	0.07
Number of dead in shell eggs	14%	0.96
Number of fertile eggs	11%	0.14
Number of infertile eggs	17%	0.37
Hatchability	12%	0.02
Chick production	19%	0.04
Time to lay	-6.1%	0.20

Conclusion

The study indicated clear breed differences in mature size, with the ZB and KR outperforming their SAB contemporaries. It also confirms results that the SAB is superior in reproductive performance compared to the ZB breed. Although it could not be substantiated from the literature the SAB also outperformed the KR as far as reproduction was concerned in the present study.

Crossbred performance indicated limited levels of heterosis for live weight and body measurements involving crosses between the SAB and ZB breeds. In contrast, the study indicated substantial levels of maternal heterosis for most reproductive traits. Benefits of crossbreeding have often been reported for fitness traits in other species, so these results are not unforeseen. It is anticipated that similar results may accrue for crossbred combinations between the KR and SAB.

This study aids in a better understanding of breed effects, and the combinability of different ostrich breeds in crossbreeding programmes aimed at commercial production. From the present results it seems coherent to combine the higher reproduction of the SAB (as a maternal line) with the improved growth of the ZB and KR (which shows more potential as terminal sires). Additional benefits to commercial production that could also be expected from such a crossbreeding programme involving the ZB male used on SAB females, is heterosis for growth to slaughter, as well as for early chick survival (Engelbrecht *et al.,* 2008).

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Chapter 6

The determination of genetic diversity between and within three ostrich subpopulations using microsatellites

Abstract

A genetic diversity study was performed on three ostrich breeds available for commercial production in South Africa to determine whether significant genetic differentiation occurs between them. The deoxyribonucleic acid of the South African Black (SAB, n = 30), Zimbabwean Blue (ZB, n = 32) and Kenyan Redneck (KR, n = 17) birds were assessed for genetic differences using 19 microsatellite loci. The number of alleles, as well as observed and expected heterozygosity of alleles was determined, as well as the genetic differentiation measured using the F-statistic and Nei's genetic distance. Significant differences were observed between the three breeds. The SAB and ZB (Fst = 0.10 and Nei = 0.49) were genetically most similar, whereas the genetic distance between the KR and ZB breeds were furthest (Fst = 0.13 and Nei = 0.61). The SAB breed exhibited the highest heterozygosity within its population and the ZB the lowest heterozygosity. Based on these results, it was suggested that crossbreeding among these breeds would lead to heterosis in commercial ostrich enterprises.

Introduction

The three ostrich breeds currently farmed with in South Africa include the South African black (SAB), Zimbabwean blue (ZB) and the Kenyan Redneck (KR). These breeds have been crossed haphazardly without proper breeding goals in the international ostrich industry, and no scientific data are available to base crossbreeding decisions upon (Petite & Davis, 1999). The SAB is a hybrid breed resulting from crossing the North African ostrich (*Struthio camelus camelus*) with the South African ostrich (*S. c. australis*) to improve feather production in the early 1900's (Deurden, 1913). The ZB (125 kg) and KR (135 kg) are reported to achieve heavier live weights than the SAB breed (115 kg; Jarvis, 1998), with the ZB exhibiting a lower reproduction performance than the SAB (Brand *et al.,* 2005; Cloete *et al.*, 2008). Phenotypical differences indicative of genetic differentiation were observed between these three subspecies. However it has not been confirmed to what extent these phenotypic differences are associated with known genetic markers.

Genetic differentiation between subpopulations can be quantified by the use of molecular markers, such as microsatellites. Microsatellites were commonly applied in genetic diversity studies in poultry and livestock species (Li *et al*., 2004; Zhou *et al*., 2005; Muchadeyi 2007; Vicente *et al*., 2008; Mtileni *et al.*, 2010). Various molecular markers have specifically been used to investigate genetic diversity between ostrich subpopulations. These markers include restricted fragment length polymorphisms (Freitag & Robinson, 1993), DNA fingerprinting (Kawka *et al.*, 2007) and microsatellites (Kimwele *et al.*, 1998; Kumari & Kemp, 1998; Kawka *et al.*, 2007). Genetic differences have been found between the KR and the Somalian Blue ostrich (Kumari & Kemp, 1998), and between the SAB, ZB and KR (Kawka *et al.*, 2007) using RFLP markers and microsatellites respectively. However these studies had small population sizes which were assessed with as few as five microsatellite markers. It is therefore necessary to confirm these results using a larger sample size and additional microsatellite markers. Microsatellites have also been used to construct a

preliminary genetic linkage map of the ostrich (Huang *et al.*, 2008). This genetic map can be of benefit to the future identification of genes affecting quantitative traits like growth, reproduction and disease susceptibility.

Genetic differentiation between and within subpopulations can be measured by the F statistic (Hartl & Clark, 1997; Holsinger & Weir, 2009). The F-statistic is directly related to the variance in the allele frequency among populations, and to the degree of resemblance among individuals within populations (Holsinger & Weir, 2009). Therefore the F-statistic will be applied in this study as measure of genetic differentiation, to determine the genetic distances between the three breeds (SAB, ZB and KR). As it is known that the crossing of breeds that are genetically more diverse from each other are expected to result in heterosis (Pirchner, 1983). Heterosis leads to improvement of lowly heritable fitness traits, like survival and reproduction, as a result of non-additive genetic variation (Pirchner, 1983). This was proved to be the case when the reciprocal crossing the SAB with the ZB led to an enhanced survival rate of crossbred chicks compared to the mean parental value (Essa & Cloete, 2006; Engelbrecht *et al.*, 2008).

Thus the aim of this study is to determine whether there are significant differences between and within the three ostrich breeds that are currently farmed with in South Africa. The results from the study could assist in finding the most suitable combination of these breeds for attaining a maximum level of heterosis.

Materials and Methods

Experimental population and locality

Blood samples were collected from 31 SAB, 35 ZB and 17 KR birds from the Ostrich population at the Oudtshoorn Research Farm. The blood samples of the SAB and ZB breeds were randomly selected from mature males and females present in the breeding flock during 2008. In contrast, the 17 blood samples that were available from the KR represented the entire genetic resource available. The resource population was developed through the donation of 76 SAB breeder birds by 61 local producers in 1964. In the 1990's more SAB birds were added to the flock and they were divided into two breeds, namely the commercial and feather breed (Bunter, 2002). According to the Bunter (2002), no evidence of heterosis was evident in the crosses between these lines and they were subsequently treated as a single genetic resource population. During 2003, 55 ZB breeding birds were added to the flock after being obtained from two local producers. These birds were originally from the Bulawayo and Harare area of Zimbabwe. Possible genetic relationships among the base population of ZB birds as well as their exact ages were unknown, although their progeny were pedigreed. Nineteen KR birds (13 males and six females) of known ancestry were introduced to the flock. The known pedigrees assisted in the choosing of birds that were not related for at least the last generation to represent this breed.

Blood collection and DNA extraction

Birds were bled from the wing vein to collect at least 2 ml of blood in EDTA vacutainer tubes. The blood samples were stored at -4°C. DNA was extracted using Proteinase K digestion standard phenol/chloroform/isoamylyl alcohol extraction procedures and absolute ethanol precipitation according to procedures described by Sambrook & Russell (2001).

Microsatellite Analysis

DNA was quantified by through spectrophotometry and gel electrophoresis. The PCR was carried out in a total volume of 5 µl comprising of 20 ng/µl of template DNA, 0.4 µM or 0.6 µM of each primer, 1 X Amplitaq goldmix or KAPA @G Fast Hotstart mastermix (KAPA Biosystems™). One primer of each locus was labelled with PET, 6-FAM, NED and VIC fluorescent dye according to Applied Biosystems™ (ABI). The PCR program was adopted from the AmplitaqGold PCR Master Mix protocol guide. After the exhaustion of the Amplitaq Gold, the KAPA 2G hotstart readymix were used. The PCR conditions for the Amplitaq Gold were five minutes denaturation at 95°C, followed by 30 cycles of denaturation at 95°C for 15 seconds. Annealing occurred at 50-70°C for one minute, depending on the annealing temperature of each primer, thereafter elongation at 72°C for one minute, ending with one cycle for the final elongation at 72°C for one minute. The PCR conditions for the KAPA 2G were denaturation at 95°C for three minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 72°C for 15 seconds and elongation at 72°C for five seconds, ending with one cycle for the final elongation at 72°C for ten minutes. Refer to appendix A for additional details of the PCR analysis.

The DNA was amplified with 23 microsatellites primers. The microsatellite primers were selected out of the studies by Kimwele *et al.* (1998), Kawka *et al.* (2007), Tang *et al.* (2003) and Huang *et al.* (2008) (Table 6.1). The Fluorescent PCR products were separated on a 6% denaturing polyacrylamide gel by using an ABI 3100 Genetic Analyzer (Applied Biosystems®) available at the sequencing facility at Stellenbosch University. The alleles were scored using ABI Prism ® Genemapper software Version 3.0 (Applied Biosystems®).

Amplification was successful for 21 of the 23 microsatellites that were tested initially. CAU42 and LIST0011 failed to amplify and were therefore excluded from all further analysis. The CAU133 and VIAS-0S14 microsatellites were also excluded from the analysis because they were monomorphic in all the samples that were tested. The remaining 19 markers were therefore used further in the statistical assessment of the data. Information on these markers is listed in Table 6.2. Three individuals, two of the SAB breed and one of the ZB breed were also omitted from the data, as many of the markers failed to amplify in DNA samples obtained from these animals. This might be due to poor DNA quality or insufficient primer binding sites during the PCR reaction.

Table 6.1 Description of microsatellite markers that were used in this study.

Marker	Tm	Sequence	Dyes	Alleles	Repeat sequence	Bp Range
CAU3	58.5	F: AACTAAGTATAGCCCTGTTACA R: TGCGAGTCTTTCTAGTTCTAC	VIC	6	$(CA)_9$	115-125
CAU14	58.5	F: ATTTAACTTCTCTAAGGCACTC R: GAGGAGCAATTCAGACAGAC	6-FAM	14	$(CA)_{16}$	142-178
CAU17	58.5	F: CGTAAACCCAGATAATCACAA R: AGTGGCATTGTAGCTCTTCA	NED	11	$(CA)_{22}$	160-180
CAU42	61.5	F: AGTCCAGCCCGCATACAC R: CCTCTGTGGAGAGAACTGTGTG	PET	7	(CA)10	182-198
CAU83	68.5	F: AAACAAGCCGCTAGTGAGGA R: TGCAGACTCAGACCAGCATC	PET	8	$(AC)_{16}$	198-218
CAU85	60.5	F: GAGGTGCCTGTCTTGTTTAC R: AAAAGCACCTTCCCACATTG	NED	16	$(AC)_{26}$	204-276
CAU128	64.9	F: TAAACACAAACAGACACAGAC R: TAACTTTGTGGCAACCAGTAG	6-FAM	4	$(AC)_{11}$	211-231
CAU129	67.9	F: GGCACAATTTCCTACCAAGC R: GGGACTGATGCTGTCTGGTT	PET	11	$(AC)_{22}$	208-236
CAU131	64.9	F: CCAATTCCGTGCATATGTGT R: TGTCAGGTGTTTCTGCATCA	VIC	10	$(CA)_{20}$	103-125
CAU133	60.7	F:GGAAGATCCTTGCTGTTGGT R: TGGACTGTTATCTGGCGATG	6-FAM	7	(CA)15	189-201
CAU144	60.7	F: ATATGCATGTGAGTATAAACAC R: CTGGGGAGCAGAGTCACC	PET	10	$(AC)_{17}$	146-167
LIST005	55	F: ATGGTGCTTTCCAGTGGTGTGC R: CATTGACCCAGGCAAGAAATCC	6-FAM	10	$(TG)_2CG(TG)_{10}$	197
LIST009	55	F: CATTGCAAACACTCTGCTGC R: TGAACGACAGGGTTATTGGC	6-FAM	13	$(CA)_{14}CG(CA)_3CG(CA)_3$	199
LIST0011	58	F: ACTGAAGTTTCCTTCTCCCC R: TTCCTGAAGCAACCACAC	PET	10	(GT)24	135
OSM1	57	F: AATCTGCCTGCAAAGACCAG R: TCCCAGTCTTGAAGTCAGCA	6-FAM	9	$(CA)_{17}$	110
OSM2	57	F: AAGCCACGGCAATGAATAAG R: CCTCAACCATTCTGTGATTCTG	NED	6	$(CA)_{22}$	121
OSM3	57	F: ATCTCCTTTGCTGGTGCAAT R: CCGGGGGGATTTCTTATGT	VIC	4	$(CA)_{15}$	157
OSM4	56	F: ATCACTTTGCTGAAGTCAAAGG R: CTAACAGAGATCTGGGCGGA	PET	5	$(CA)_{16}$	134
OSM5	59	F: GTGGATCAGTTCAATCCTTGC R: GCCCAAGAAAATGATGGAGA	NED	6	$(CA)_{20}$	232
OSM7	58	F: AGCATACACATGCAGACCCC R: TGTTTCCTGCCATTCTGTCA	VIC	7	$(CA)_{16}CT(CA)_5CT(CA)_{25}$	215
VIAS-OS4	53.7	F: CTCCTGGATGTTCTAGCAGT R: CTCCTTGTCCAGCCATATAC	VIC	12	$(GTGTAT)_2(GT)_9$	216-268
VIAS-0S14	49.9	F: CACTTCTCCGAATTTTAAAAGG R: AGGAAGAGATGTGGAGTCCC	6-FAM	18	$(AC)_{21}$	209-245
VIAS-OS29	55.1	F: TTTTCGTCTTCCACCCACTG R: CTGCTTCTTCCGTGTGTGTC	PET	18	$(AC)_{13}GG(AC)_6GG(AC)_4$	123-173

Statistical analysis

The 19 remaining microsatellites were checked for null alleles or scoring errors by the software program Microchecker 2.2.3. After this check the microsatellite loci were evaluated for possible signs of selection using the Fst-outlier method of Beaumont & Nichols (1996) implemented in Lositan Version 1 (Antao *et al.,*

2008), Genetix 4.0.5.2 were then run to test for the number of alleles as well as the expected and observed heterozygosity for each microsatellite.

CAU144 and LIST005 were found to possibly contain null alleles and four markers were under positive selection at the 95% confidence level. The latter four markers were VIAS-014, CAU3, OSM4 and LIST009. The analysis was performed without the four markers appearing to be subjected to positive selection. Analysis was also performed excluding the two markers containing null alleles.

The genotypic and allelic frequencies were estimated using the software program GDA Version1.1 (Weir, 1996) and microsatellite toolkit analysis were used to determine the average number of alleles, expected and observed heterozygosity and fixation index with the respective standard deviations for each subpopulation. The fixation index is a measure of the excess homozygosity within a population and is interrelated to the inbreeding coefficient (Hamilton, 2009). Genetic differentiation was measured in terms of pairwise Fst values calculated in Genetix Version 4.0.5.2. The Nei's statistic was calculated using GENEPOP software (Raymond & Rousset, 1995).

Results and discussion

Genetic diversity

The four markers which were under positive selection were excluded from the analysis and it did not affect the results of whether there are genetic differences between the breeds significantly. No differences were observed in terms of genetic differentiation between the three breeds using either 15 or 19 markers. The results for the microsatellite marker polymorphisms are shown in Table 6.2. All 19 markers used were polymorphic. A high number of alleles of 28 were observed for CAU85 in contrast to the number of alleles (16) that were reported previously (Tang *et al.*, 2003). The microsatellite marker, LIST009 had 27 alleles in comparison to the 13 number of alleles reported before (Kumari & Kemp, 1998), while the number of alleles observed for OSM7 were 24 *vs.* the seven alleles reported by Kimwele *et al.* (1998). These differences in number of alleles may be due to allele-drop out, which was not observed in the abovementioned studies, because of fewer PCR cycles during their annealing stage. In the present study more PCR cycles were used for the amplification of the DNA, thus causing more alleles to be observed. The genetic diversity is described by the mean number of alleles per locus as well as the mean expected and observed heterozygosity of those alleles. A total of 263 alleles were observed across the three ostrich breeds. The average number of alleles per locus was 13.8 (Table 6.2). The expected heterozygosity amounted to 0.81 and the observed heterozygosity 0.69 for all the loci across all three breeds. This implies that there is a substantial amount of genetic diversity within the three ostrich breeds farmed with commercially in South Africa.

Table 6.2 Observed results for microsatellite markers across the South African Black (SAB), Zimbabwean Blue (ZB) and Kenyan Redneck (KR) ostrich breeds.

Marker	Alleles	Observed size	Expected size	He	Ho	Fi
CAU3	5	120	125	0.67	0.63	0.07
CAU14	10	155	178	0.85	0.83	0.03
CAU17	12	197	180	0.86	0.72	0.20
CAU83	8	217	218	0.76	0.58	0.30
CAU85	28	287	276	0.95	0.92	0.03
CAU128	8	223	231	0.61	0.61	0.01
CAU131	12	120	125	0.81	0.72	0.11
CAU133	4	196	201	0.71	0.71	0.01
CAU144	9	166	167	0.74	0.45	0.40
LIST005	16	224	197	0.88	0.63	0.30
LIST009	27	328	199	0.94	0.80	0.20
OSM1	16	141	110	0.84	0.80	0.06
OSM2	17	187	121	0.90	0.66	0.30
OSM3	7	152	232	0.59	0.58	0.03
OSM4	13	159	134	0.83	0.55	0.3
OSM7	24	241	215	0.93	0.89	0.04
VIAS-0S4	14	272	268	0.80	0.53	0.3
VIAS-OS14	16	243	245	0.88	0.74	0.2
VIAS-0S29	17	155	173	0.89	0.88	0.01
Average	13.8±1.6			0.81±0.2	0.69±0.03	0.15±0.03
Total	263					

*He- expected heterozygosity

*Ho-observed heterozygosity

*Fi- fixation index

An average of 8.1 alleles was found across the three breeds, with a mean expected heterozygosity of 0.74 and an observed heterozygosity of 0.69. Observed heterozygosity was the highest in the SAB (0.72 ± 0.019) subspecies, whereas it was the lower for the ZB (0.68 ± 0.019) and KR (0.68 ± 0.026) breeds. These results are consistent with those of Kawka *et al.* (2007), who also found that the SAB breed had the highest heterozygosity and the KR the lowest. This may be because the SAB is a composite breed derived from the North African (S. *c. camelus*) and South African (*S. c. australis*) ostriches (Deurden, 1913). The SAB genetic resource located on the Oudtshoorn Research Farm was also obtained from different sources of origin (Bunter, 2002), possibly contributing to a higher diversity pool for this breed. Heterozygosity in the ZB was lower than expected, due to the presence of more homozygotes in the ZB population than expected. This trend towards lower genetic variability can possibly be attributed to the fact that no prior knowledge was available of possible kinship relationships between individuals within the ZB breed. It could thus be possible

for these birds to be related to each other. This can explain the closer relatedness, as reflected by the higher fixation index value observed for this breed.

Table 6.3 Mean (± SD) number of alleles, expected heterozygosity (He), observed heterozygosity (Ho) and fixation index (Fi) for the South African Black, Zimbabwean Blue and Kenyan Redneck breeds.

Population	Alleles	He	Ho	Fi
SAB	8.8 ± 4.06	0.75 ± 0.030	0.72 ± 0.019	0.05
ZB	9.4 ± 3.96	0.78 ± 0.034	0.68 ± 0.019	0.13
KR	6.2 ± 2.29	0.69 ± 0.024	0.69 ± 0.026	0.01
Average	8.1	0.74	0.69	0.07

*He- expected heterozygosity

*Ho-observed heterozygosity

*Fi- fixation index

Genetic differentiation between breeds

Significant differences were observed in terms of the genetic resemblance between the three breeds. The SAB and ZB were genetically more similar to each other (Fst = 0.10 and Nei = 0.49). The largest genetic distance were estimated between the ZB and KR breeds (Fst = 0.13 and Nei = 0.61). The latter result is unexpected, because the areas of origin of the ZB and KR breeds are geographically closer situated than those of the SAB and KR. In the literature, the largest genetic distance was previously reported between the SAB and KR breeds, and the smallest between the ZB and KR breeds (Kawka *et al.*, 2007). The inconsistency of results might be attributed to the fact that a bigger population size and a wider variety of microsatellites were used in this study when compared to the study of Kawka *et al.* (2007). An Fst value of zero implicates that the variance of the allele frequencies within each population is similar (Hartl & Clark, 1997; Holsinger & Weir, 2009). All these F-statistic values falls in the range of 0.05 to 0.15 which are interpreted as moderate genetic differentiation between the breeds (Hartl & Clark, 1997), and therefore the variance of the allele frequencies between the SAB, ZB and KR differs. The genetic differentiation of the three breeds is illustrated in Figure 6.1.

Table 6.4 Mean Nei's (Nei, 1972) standard genetic distances, pairwise F-statistic (Weir & Cockerheim, 1984), value as a measure of genetic variation between the South African Black (SAB), Zimbabwean Blue (ZB) and Kenyan Redneck (KR) ostrich breeds.

Breeds	Nei's genetic distance	Pairwise F-statistic
SAB and ZB	0.49	0.10
SAB and KR	0.51	0.12
ZB and KR	0.61	0.13

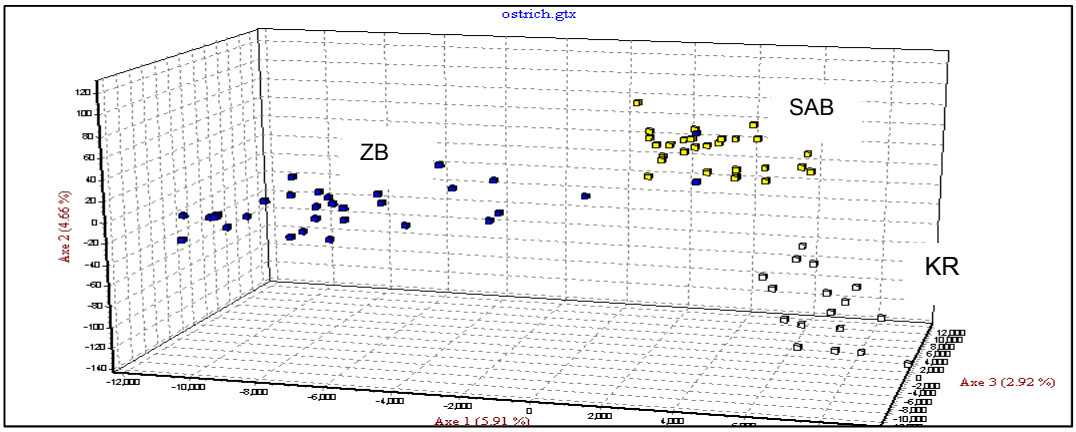

Figure 6.1 Three dimensional graph depicting the genetic differentiation between the SAB, ZB and KR breeds.

Conclusion

The study indicates considerable genetic differentiation between the three ostrich breeds considered. The SAB breed seems to have the highest level of genetic variation, and genetic improvement has been achieved by selecting for chick production in this breed. The SAB founder population has also been sourced from different origins and different lines (commercial *vs.* feather) to form the basis of the population represented in this study. The ZB and KR exhibited the lowest levels of genetic variation. In the case of the ZB breed, this result may stem from the fact that the resource population did not have any ancestrial information albeit sourced from three separate commercial entities. This argument is supported by a higher fixation index, hinting at a closer relationship between the individuals. Based on the fixation index, the same argument cannot be applied to the KR breed although all animals originated from the same property. However, the low levels of genetic variation within the ZB and KR breeds can be seen as a benefit, as crossing of separate populations with a higher level homozygosity is expected to leads to increased levels of heterosis. The genetic differences obtained between the SAB, ZB and KR serves as confirmation of the phenotypic differences reported between the breeds. Application of this knowledge can lead to economically viable commercial crossbreeding programs based on scientific principles. Hybrid vigour stemming from crossbreeding can lead to improved reproduction of crossbred females, as well as an improved survival of crossbred chicks. Since these improvements stem from the non-additive part of genetic variation, it is likely to be well adapted to commercial situations where terminal crossbreeding systems are applied.

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Chapter 7

General conclusions

The aim of this study was to characterize the differences between the three ostrich breeds that form the genetic resource for the South African ostrich industry, namely the South African Black (SAB), Zimbabwean Blue (ZB) and the Kenyan Redneck (KR) in relation to growth, carcass and reproduction traits. Microsatellites were furthermore used to determine the genetic differentiation between and within these three breeds. The need for the abovementioned aim arose from the uncontrolled and sometime haphazard random crossing of ostrich breeds in the local and international industries. It was argued that information about the characteristics of each breed will provide the knowledge required for the implementation of sound crossbreeding systems.

The KR had the fastest growth rate and attained a higher mature live weight than the SAB breed. The ZB breed grew slower than the KR breed, but attained a similar mature live weight as the KR. Based on this information, it was argued that the ZB breed are relatively late maturing compared to the other breeds. Crossing of the SAB and ZB breeds resulted in significant heterosis for the growth parameters of the Gompertz curve, as well as for specific weights that could serve as an indication of growth. Crossbreds showed inconsistent results, with the SAB x ZB cross resembling the SAB breed for mature live weight, while the ZB x SAB breed resembled the ZB breed for mature live weight. These inconsistent results might be due the small number of the crossbred breeds (particularly the SAB x ZB combination) being represented in the dataset. Further analysis, using more evenly distributed sample number of all the breeds is required to verify the abovementioned results, and to derive definite recommendations regarding the growth of crossbred breeds.

Differences were observed between the three breeds for carcass and slaughter traits. The ZB breed outperformed their SAB contemporaries for slaughter weight. The KR reached a similar slaughter weight to that of the SAB breed. The latter result may be due to the KR breed being underrepresented in the dataset. In contrast, dressing percentage were the highest for the KR breed, with SAB birds having a low dressing percentage. Samples from the SAB exhibited better meat quality traits than the meat samples from the ZB and KR breeds, in terms of having more favourable means for pH, cooking loss percentage and meat colour traits. The crossbred progeny resembled the ZB and KR breeds for carcass and slaughter traits and resembled the SAB breed for meat quality traits. Crossbred progeny thus seems able to realise a higher quantity of product, while maintaining a meat quality similar to that of the SAB breed.

Reproduction of purebred SAB females was confirmed to be superior to that of their ZB and KR contemporaries. The reciprocal cross between the SAB and ZB breeds was subjected to substantial levels of maternal heterosis, amounting to 12% for egg production and 19% for chick production. These results suggest that the SAB breed is combinable with the other breeds. Commercial production may thus seek to

combine the high reproduction of SAB females with the superior size (ZB) and dressing percentage (KR) of the other breeds. Crossbred chicks are also expected to benefit from direct heterosis for chick survival, which is universally acknowledged as a constraint to efficient ostrich production. The potential benefit of heterosis for chick survival were found significant (Essa & Cloete, 2006; Engelbrecht *et al.,* 2008).

Significant differences were also observed between the three breeds on a molecular level. The KR breed was found to be genetically more distinct from both the SAB (Fst = 0.12 and Nei = 0.51) and ZB (Fst = 0.13 and Nei = 0.61) breeds. The genetic distance between the SAB and ZB breeds were closest (Fst = 0.10 and Nei = 0.49). Genetic variation was the highest within the SAB breed, and the lowest within the ZB and KR breeds, observed heterozygosity values amounting to respectively 0.72, 0.68 and 0.69. Based on these results, genetic distances between the three breeds were thought to be sufficient to support substantial levels of heterosis in crossbreeding systems.

The results from this study can be used to support more goal orientated ostrich crossbreeding systems for commercial production. It has been established that the SAB breed exhibits more efficient reproductive performance with enhanced meat quality traits. In contrast the ZB and KR lines exhibited heavier mature live weights, with the KR having the fastest growth rate and the ZB reaching maximum growth at a later stage. Genetic differences on the molecular level were also observed between the three commercial ostrich breeds. From these results, commercial ostrich farmers may seek to exploit the combinability of ostrich breeds, while also benefiting from advantages due to heterosis.

The general recommendation from this study is that the SAB breed may be used as a dam line because of its superior reproductive ability and improved meat quality traits. In contrast, the ZB and KR may be used as sire lines because of their superior growth and carcass traits in a specific crossbreeding system, and ability to produce heavier crossbred slaughter birds with meat quality attributes similar to that of purebred SAB birds. The expression of individual heterosis in the above crossbreeding system can be exploited to produce heavier slaughter birds over a similar rearing period as SAB birds, but with favourable carcass and meat quality traits.

It needs to be conceded that there are still issues pertaining to the usage of the ostrich genetic resource that need to be resolved. Some crossbred combinations for which information is required were not represented, while the KR breed was represented by too few individuals. Further investigation is therefore necessary, using a larger dataset which is representative of all the purebred breeds (SAB, ZB and KR) as well as their reciprocal crossbred progeny. Such studies are anticipated to produce more accurate results for individual and maternal heterosis, as well as more comprehensive information of the strong and weak points of each breed and their crossbred combinations with a commercial application. Further studies should investigate the impact of breed and crossbred combination upon skin and feather traits. The emphasis on meat in the present study is validated by the commanding position of meat as a commodity in the income derived from slaughter birds at present. However, it should be considered that the skin and feathers also contribute to the

income of commercial ostrich farmers. No comprehensive crossbreeding strategy could thus be complete before this information is gathered and included in the overall strategy.

Appendix A

Table 6.5 PCR reagents used during this study.

PCR reagents	Volume	Final concentration
dH_2O	1.1/0.9	
Forward primer (10nmol)	0.2/0.3	0.4uM/0.6 uM
Reverse primer (10nmol)	0.2/0.3	0.4uM/0.6 uM
DNA template	1ul	20ng/ul
Amplitaq Gold Mix/ KAPA 2G Fast Hotstart (X2)	2.5ul	1X
Total volume	5ul	

Table 6.6 Description of the PCR program adopted from Amplitaq Gold.

Function	Temperature	Time	Cycle
Denaturation	95ºC	5 min	X1
	95ºC	15 sec	
Annealing	50-70 ºC	1 min	
Elongation	72 ºC	1 min	X30
Final Elongation	72ºC	7 min	X1

Table 6.7 Details pertaining to the PCR program for KAPA 2G Fast Hotstart Readymix.

Function	Temperature	Time	Cycle
Denaturation	95°C	3min	X1
	95°C	15 sec	
Annealing		15sec	X40
	72°C	5sec	
Elongation	72°C	10min	X1