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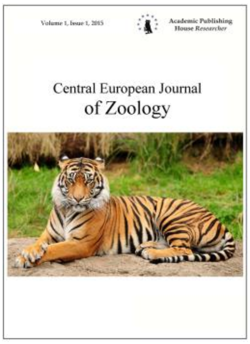

Articles

Early Thermal Acclimatization in Pre-Starter and Starter Chicks Fed Varying Crude Protein Diets Fortified with Optimum Electrolyte Balance

Ibukun Olukorede Popoola [a,b,*], Oluwabukola Rashidat Popoola [a,b], Ibikunle Funso Olaleru [a,c], Ibukun Oluwatobi Busari [a], Femi Joshua Oluwadele [a,d], Oluwaseyi Olamide Olajide [a]

[a] University of Ibadan, Oyo State, Nigeria
[b] Thisavrous Pyrgos Int. Resources, Oyo State, Nigeria
[c] National Root Crops Research Institute, Umudike, Nigeria
[d] Federal University of Oye Ekiti, Ekiti State, Nigeria

Abstract

The genetic potentials of fast-growing strains of broiler chickens have been compromised as a result of the huge contribution of external factors determining the expression of these useful traits. The earth`s present surface temperature has necessitated adjustments in major contributing factors to beneficial traits expression in broilers that includes nutritional balance in the face of thermal oscillation. Crude protein (CP) ingestion cause a greater increase in heat production than carbohydrates or fat. However, controversy abounds on whether or not low CP diets are beneficial to poultry raised in hot environment. It is essential to investigate the effects of heat stress acclimation during early weeks on adaptability to high endogenous heat production in broiler chicks. Using a total of 288, one day-old Arbor Acre broiler chicks, birds were randomly allotted to four dietary treatments (T1 – 23 % CP; T2 – 21 % CP; T3 – 19 % CP; and T4 – 17 % CP) with six replicates, in a completely randomized design. Data were analysed using descriptive, ANOVA, regression and correlation statistics. Pre-starter chicks cannot tolerate excess endogenous heat load from protein degradation and therefore divert dietary proteins needed for growth into regulation of homeostasis. Heat-stressed starter broiler chicks can tolerate excessive endogenous heat load at optimum DEB of 270 mEq/kg and achieve a new energy and homeostatic balance. However, production and economic prowess necessitate the adoption of 21 % CP in diets of pre-starter and starter broiler chicks under severe heat stress conditions.

Keywords: broiler chicks, crude protein, endogenous heat, heat stress, protein efficiency.

1. Introduction

The metabolism of crude protein ingested by livestock results in a greater increase in heat production than carbohydrates or fats (Musharaf, Latshaw, 1999). Zaman et al. (2008) reported that a reduction of crude protein in rations fed to broilers under heat stress condition, with adequate amount of essential amino acids (EAA) may improve performance. According to Cheng et al. (1997), the provision of higher crude protein in diets of heat-stressed broilers, in attempt to

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[*] Corresponding author
E-mail addresses: popoolaibukun@yahoo.com (I.O. Popoola)

compensate for low appetite, is detrimental to production parameters. However, contrasting reports were documented by Temim et al. (2000), affirming that low-CP diets are not beneficial to poultry under heat stress conditions.

Similarly, Awad et al. (2014) reported a decrease in performance of broilers fed diets with low CP, even when all essential amino acid requirements are met. Si et al. (2004) also noted reduced appetite in birds fed low-CP diets. Although, Soleimani et al. (2012) opined that living organisms respond to thermal stressors by synthesizing a group of highly conserved proteins known as heat shock proteins as they function in modifying physiological stress response and stress tolerance acquisition. Buyse et al. (1992) noted reduced growth performance in response to increased heat production in male broiler chickens, while affirming that the heat increment in broilers fed a lower protein diet was as a result of elevated plasma triiodothyronine (T3) concentration, which may consequently increase heat production.

The trending prediction is that the production cycle of broiler chickens which has been decreased over the years will yet more decrease due to heat stress conditions, as Popoola et al. (2020a) expansiated that even though ideal dietary electrolyte balance is essential for heat-stressed broilers, yet salts that presumably could have improved satiety did not enhance appetite in heat-stressed broilers, necessitating a more integral nutritional approach. Also, poultry meat and feed quality deterioration; and loss of customers` preference to chicken meat exposed to heat stress conditions may persist as noted by Popoola et al. (2020b). According to Nichelmann and Tzschentke (2002), early post-hatch period is more important where major developmental and physiological processes occur. Although, chicks anatomically seem complete post-hatch, yet some systems such as digestive, immune, and thermoregulatory systems need further development and maturation for optimum performance. Adaptation to heat stress conditions can be enhanced by thermal conditioning in chicks, without impairing performance (Decuypere et al., 2001), as chicks regulate body temperature during early post-hatch periods more with proportionate increase in age (Debonne et al., 2008).

Ali and Hossain (2010) stated that heat stress in broilers can be reduced by early-age thermal conditioning, as it is a sensitive process of induction of thermo-tolerant traits in immature neonatal chicks at an early age, by developing the hypothalamus, which is the thermo-regulatory centre, and has been proven to be the most appropriate age to exploit and induce thermotolerance in broiler chickens (Yahav et al., 2005). Tan et al. (2010) noted that heat stress during early post-hatch periods may result in greater adaptability to thermal stress even when endogenous heat production is higher. Since birds must maintain internal body temperature despite thermal oscillation, they do so at the expense of production, by diverting nutritionally beneficial molecules to homeostatic adjustments. Popoola et al. (2020a) affirmed that tropical regions with characteristic high environmental temperatures face more difficulty raising fast-growing meat type chickens because birds` health, performance and meat quality are adversely affected. The level of heat tolerance in poultry is dependent on the activation of heat loss mechanism and the ability to reduce the endogenous heat production (Nichelmann, Tzschentke, 2002). Also, Popoola et al. (2020c) noted that ideal DEB is prerequisite to blood acid-base balance and reduced incidences of hemodilution in heat-stressed broilers. Azad et al. (2010) noted that heat stress affects metabolic processes, causing oxidative damage to skeletal muscles with impairment of functional properties of meat. Similar reports were documented by Barbut (1997) noting that pale, soft, exudative meat resulted from broilers exposed to heat stress. Mccurdy et al. (1996) explained that rapid post-mortem glycolysis decreases pH of meat at slaughter and results in muscle protein denaturation.

For fast-growing meat type chickens, the efficiency of the lungs further determine physiological responses to heat stress conditions, as the capacity of the lungs to always meet oxygen demands needed for survival, growth, and production is not always met, thereby impairing the ability to regulate energy balance, because the lungs grow less rapidly than the rest of the body as evidenced in hypoxia and ascites (Luger et al., 2003). Early thermal manipulations in poultry may be useful in tropical regions with more vulnerability to heat stress, when the chicks` body temperature and feedback regulatory mechanisms are immature (Yahav, 2000). Nascimento et al. (2011) reported that higher sensible heat loss may be beneficial as to promote heat tolerance in broilers compared to evaporative heat loss that may lead to dehydration.

According to Yardimci et al. (2006), weight gain was unaffected in broilers that were earlier conditioned thermally, because an enhancement was observed in the regulatory capacity of the chicks to extreme environmental temperatures. Ozkan et al. (2010) noted that low ambient temperature triggers an increase in heat production, and oxygen requirement of the body. Furlan et al. (2002) also affirmed that chicks exposed to cold stress in the first week post-hatch often suffer from hypothermia with characteristic symptom of pulmonary hypertension that can reduce farmers` profit. Heat stress acclimation involves a shift in energy balance from an unstable state to a more physiologically stable state of homeostasis under recurrent thermal oscillation.

According to Leterrier et al. (2009), the growth of broilers have been improved by genetic selection. However, some visceral organs have not been genetically modified alongside traits of most importance, thereby creating a negligence in effective capacity of cardiovascular and respiratory systems for hyperventilation and heat loss. Yahav and McMurtry (2001) reported that pre-starter exposure of chicks to heat stress enabled them to withstand heat challenge at finisher phase when metabolic processes increased, with subsequent reduction in mortality as De Basilio et al. (2003) reported that chickens that did not survive heat challenge at 34 days of age had higher body temperatures of about 0.6 °C prior to heat challenge than those of the survivors. Therefore, the effect of endogenous thermal acclimatization in the face of external thermal oscillation on performance of pre-starter and starter chicks were investigated.

2. Materials and methods

The study was carried out at the Teaching and Research Farm, University of Ibadan, Nigeria, after the experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee, through the Agricultural Biochemistry and Nutrition Unit of the Department of Animal Science. A total of 288, one day-old Arbor Acre broiler chicks with initial body weight of 41±3g were randomly allotted to four dietary treatments (CP, %: 23, 21, 19, 17) at pre-starter and starter phases with six replicate groups in a completely randomized design in order to evaluate the effects of varying dietary CP at ideal DEB on early thermal acclimatization of heat-stressed broiler chicks. Feed grade potassium chloride and sodium bicarbonate, and the inherent potassium, sodium and chloride ions in feed ingredients were the electrolyte sources computed for determining the aggregate DEB using the equations derived by Popoola and Iyayi (2018). The derived equations were based on assumptions opined by Popoola et al. (2020c) for an ideal DEB, affirming that not more than 30 to 140 mEq/kg DEB are required from mineral sources, with about 115 to 210 mEq/kg DEB obtainable from feed ingredients.

The derived equation of DEB is $\sum \text{DEB} = \sum (\text{Na}^+ + \text{K}^+) - \sum \text{Cl}^- \ldots [y]\ [c]$ (1)
Where [y] = mineral sources
and [c] = other macro ions (Ca, Mg, P, S etc) held constant.
$\sum \text{DEB} = \iota\, \text{DEB} + \varepsilon\, \text{DEB}$ (2)
Where $\sum$ DEB = Aggregate DEB; ι DEB = Inherent DEB in rations and ε DEB = DEB in Electrolyte sources.

Feed intake was determined by giving a known quantity of feed to the birds and subtracting the left over for a given period from the quantity supplied. This difference was divided by the number of birds in a replicate group to estimate the feed intake per bird. Body weight gain of birds was determined by subtracting the initial weight for each week from the final weights with the aid of sensitive weighing scale. A total of 32 pens were used in this study in order to measure water intake in heat-stressed broiler chicks as described by Popoola et al. (2019). Maximum and minimum average ambient temperature and relative humidity were monitored on a daily basis using a digital hygro-thermometer. Rectal temperature was measured in the morning (06:00-08:00 h) and afternoon (13:00-15:00 h), with the use of a digital rectal probe. Two birds per pen with body weight closest to the class mean weight were identified for body temperature measurement. Proximate analysis of the feeds was determined according to AOAC (2005) procedure. Assay was conducted for sodium and potassium (Flame spectrophotometer), and chloride (titration) in diets fed to broiler chickens at different phases of growth (Lacroix et al., 1970). Data obtained were subjected to descriptive statistics, analysis of variance using SAS package (2012), regression and correlation statistics. Means for treatments in the analysis of variance were compared using Duncan Multiple range test and all statement of significance were based on probability level of 0.05.

3. Results

The mean temperature and relative humidity observed in the pen house are presented in Table 1. The recorded values of mean temperature and relative humidity were indicative of severe heat stress environment. The analysed nutrients in diets fed to pre-starter and starter chicks were shown in Table 2. Tables 3 and 4 show the performance of heat-stressed pre-starter and starter chicks fed varying dietary crude protein at ideal DEB.

Feed intake

Feed intake (FI) of heat-stressed birds on different dietary CP varied significantly ($P < 0.05$) even at ideal DEB. FI values observed in pre-starter birds on 23 % CP (475.25), 21 % CP (454.08) and 19 % CP (436.78) were similar and significantly ($P < 0.05$) higher compared to 17 % CP (380.65 g/bird). However, at starter phase, FI of heat-stressed birds was not significantly ($P > 0.05$) affected by varying dietary CP, and values ranged from 379.67 (17 % CP) to 421.50g/bird (23 % CP).

Body Weight Gain

Body weight gain (BWG) of pre-starter chicks on 23 % CP diet (321.13g) was significantly ($P < 0.05$) higher compared to other dietary treatments. However, birds on 2 1% CP (273.03) and 19 % CP (279.45) had similar ($P > 0.05$) BWG values. Diet with 17 % CP value resulted in the lowest ($P < 0.05$) BWG in pre-starter chicks. However, at starter phase, BWG values observed in birds on 23 % CP diet (261.83), 21 % CP diet (228.11) and 19 % CP diet (222.44) were not significantly ($P > 0.05$) different. However, starter chicks on 17 % CP diet (187.16) had the lowest ($P < 0.05$) BWG.

Table 1. Mean temperature and relative humidity of the pen house

TIME (h)	Mean Temperature range (°C)	Mean relative humidity (%)
0600 – 0700	21.5 ± 2	99
0700 – 0800	23.8 ± 2	98
0800 – 0900	26.2 ± 2	96
0900 – 1000	28.8 ± 2	90
1000 – 1100	30.5 ± 2	89
1100 – 1200	33.9 ± 2	69
1200 – 1300	36.4 ± 2	58
1300 – 1400	42.4 ± 2	47
1400 – 1500	37.7 ± 2	55
1500 – 1600	34.7 ± 2	63
1600 – 1700	31.1 ± 2	87
1700 – 1800	28.9 ± 2	90
1800 – 1900	24.9 ± 2	97

Table 2. Analysed nutrients in diets fed to pre-starter and starter chicks

Nutrients (%)	23% CP	21% CP	19% CP	17% CP
Crude protein g/kg	22.87	21.10	18.80	17.20
ME, kcal/kg	3062.08	3047.30	3046.63	3081.40
Ether extract g/kg	3.88	3.83	3.80	3.82
Crude fibre g/kg	3.45	3.44	3.28	3.08
Calcium g/kg	1.00	1.03	1.00	1.04
Total phosphorus g/kg	0.80	0.74	0.70	0.69
NPP, g/kg	0.43	0.43	0.42	0.41
Ca:NPP	2.33	2.39	2.38	2.54
Sodium g/kg	0.29	0.29	0.33	0.37
Potassium g/kg	0.96	1.02	1.06	1.01
Magnesium g/kg	0.17	0.17	0.16	0.16
Chlorine g/kg	0.35	0.42	0.51	0.53

ME – Metabolizable energy, NPP – Non-phytate phosphorus, Ca – Calcium, CP – Crude protein

Feed conversion ratio and efficiency

The FCR values observed in pre-starter chicks varied significantly (P<0.05) with different dietary CP levels. The lowest (P < 0.05) FCR value was observed in 23% CP (1.47), although it did not differ significantly (P > 0.05) from 19 % CP (1.56). However, birds on 21 % CP (1.67) and 17 % CP (1.72) had the highest (P < 0.05) FCR value at pre-starter phase. At starter phase, there were no significant (P > 0.05) differences observed in FCR values of heat-stressed birds on 23 % CP (1.64), 21 % CP (1.85) and 19 % CP (1.83). However, 17 % CP diet (2.03) resulted in the highest (P < 0.05) FCR value. At pre-starter phase, 23 % CP (0.68) and 19 % CP (0.64) had similar feed efficiency. The lowest (P < 0.05) feed efficiency value was observed in 21 % CP diet (0.60) and 17 % CP diet (0.59). However, in starter chicks, feed efficiency did not differ significantly (P > 0.05) for diets with 23 % CP (0.63), 21 % CP (0.55) and 19 % CP (0.57). However, the lowest (P < 0.05) feed efficiency value was observed in 17% CP diet (0.50).

Protein Intake and Efficiency ratio

At pre-starter phase, protein intake observed in birds on 23 % CP (109.31) was significantly (P < 0.05) higher compared to other dietary treatments. Birds on 17 % CP (64.71) had the lowest (P < 0.05) protein intake, which increased with increasing dietary CP levels. However, at starter phase, there were no significant (P > 0.05) differences in protein intake values of heat-stressed birds, with respect to varying dietary CP levels.

Protein efficiency ratio (PER) in pre-starter chicks varied significantly (P<0.05) with respect to dietary CP values. It was observed that chicks on 23 % CP (3.37) and 21 % CP (3.32) had similar PER values and were significantly (P < 0.05) lower compared to 19 % CP (3.89) and 17 % CP (4.15). However, at starter phase, birds on 23 % CP (2.73), 21 % CP (2.39) and 19 % CP (2.47) had similar PER values. Birds on 17 % CP (2.17) had the lowest (P < 0.05) PER value.

Water intake and rectal temperature

Water intake of heat-stressed pre-starter chicks was not significantly (P > 0.05) influenced by varying dietary CP, and values ranged from 216.51 to 240.24mL/bird/day. However, at starter phase, birds on 23 % CP (451.77) and 19 % CP (435.23) had significantly (P < 0.05) higher water intake compared to 21 % CP (395.38) and 17 % CP (370.87mL/bird/day).

Figure 1 shows the body temperature change, morning and afternoon rectal temperature of heat-stressed broiler chicks on different levels of dietary crude protein. There were significant (P<0.05) differences observed in morning and afternoon rectal temperature of heat-stressed broiler chicks. Birds on 23 % dietary CP had the highest morning rectal temperature (RT) and differed significantly (P < 0.05) from other dietary treatments. Birds fed 19 % CP had the lowest

($P < 0.05$) morning RT. There was no significant ($P > 0.05$) difference observed in morning RT of heat-stressed birds fed 21 % and 17 % dietary CP.

For afternoon RT, a significant ($P < 0.05$) increase was observed in afternoon rectal temperature of heat –stressed birds fed 23 % CP compared to other dietary treatments. However, there were no significant ($P > 0.05$) differences observed in the afternoon RT of heat-stressed birds fed 21 %, 19 % and 17 % dietary CP at starter phase. Figure 2 shows the body temperature fluctuation in heat-stressed broiler chicks on varying levels of dietary crude protein. A significantly ($P < 0.05$) higher temperature change (TC) was observed in starter chicks fed 23 % (0.81) dietary CP and did not differ from those on 19 % CP (0.73). However, birds on 21 % CP and 17 % CP had the lowest ($P < 0.05$) TC of 0.69°C.

Table 3. Performance of pre-starter broiler chicks fed varying dietary crude protein at ideal dietary electrolyte balance

Crude Protein (%)	Mean FI (g/bird)	BWG (g/bird)	FCR (g/g)	Gain : Feed (g/g)	Protein Intake (g/bird)	PER (g/g)	Water intake (mL / bird / day)
23	475.25^{a}	323.13^{a}	1.47^{b}	0.68^{a}	109.31^{a}	3.37^{b}	240.24
21	454.08^{a}	273.03^{b}	1.67^{a}	0.60^{b}	95.36^{b}	3.32^{b}	216.51
19	436.78^{a}	279.45^{b}	1.56^{ab}	0.64^{ab}	82.99^{c}	3.89^{a}	236.61
17	380.65^{b}	222.62^{c}	1.72^{a}	0.59^{b}	64.71^{d}	4.15^{a}	218.13
SEM	18.08	7.73	0.07	0.03	3.66	0.16	8.69
P Value	0.00	0.00	0.11	0.11	0.00	0.00	0.15

^{abcd} Means of treatments along a column with different superscripts differed significantly ($P < 0.05$) using DMRT. FI – Feed intake, BWG – Body weight gain, FCR – Feed conversion ratio, PER –Protein efficiency ratio.

Table 4. Performance of starter broiler chicks fed varying dietary crude protein at ideal dietary electrolyte balance

Crude Protein (%)	FI (g/bird)	BWG (g/bird)	FCR (g/g)	Gain: Feed (g/g)	Protein Intake (g/bird)	PER (g/g)	Water intake (mL/bird/day)
23	421.50	261.83^{a}	1.64^{b}	0.63^{a}	96.95	2.73^{a}	451.77^{a}
21	413.33	228.11^{ab}	1.85^{ab}	0.55^{ab}	95.07	2.39^{ab}	395.38^{b}
19	392.50	222.44^{ab}	1.83^{ab}	0.57^{ab}	90.28	2.47^{ab}	435.23^{a}
17	379.67	187.16^{b}	2.03^{a}	0.50^{b}	87.32	2.17^{b}	370.87^{b}
SEM	23.34	18.32	0.12	0.04	5.37	0.17	24.90
P Value	0.58	0.07	0.21	0.17	0.58	0.17	0.12

^{ab} Means of treatments along a column with different superscripts differed significantly ($P < 0.05$) using DMRT. FI – Feed intake, BWG – Body weight gain, FCR – Feed conversion ratio, PER-Protein efficiency ratio.

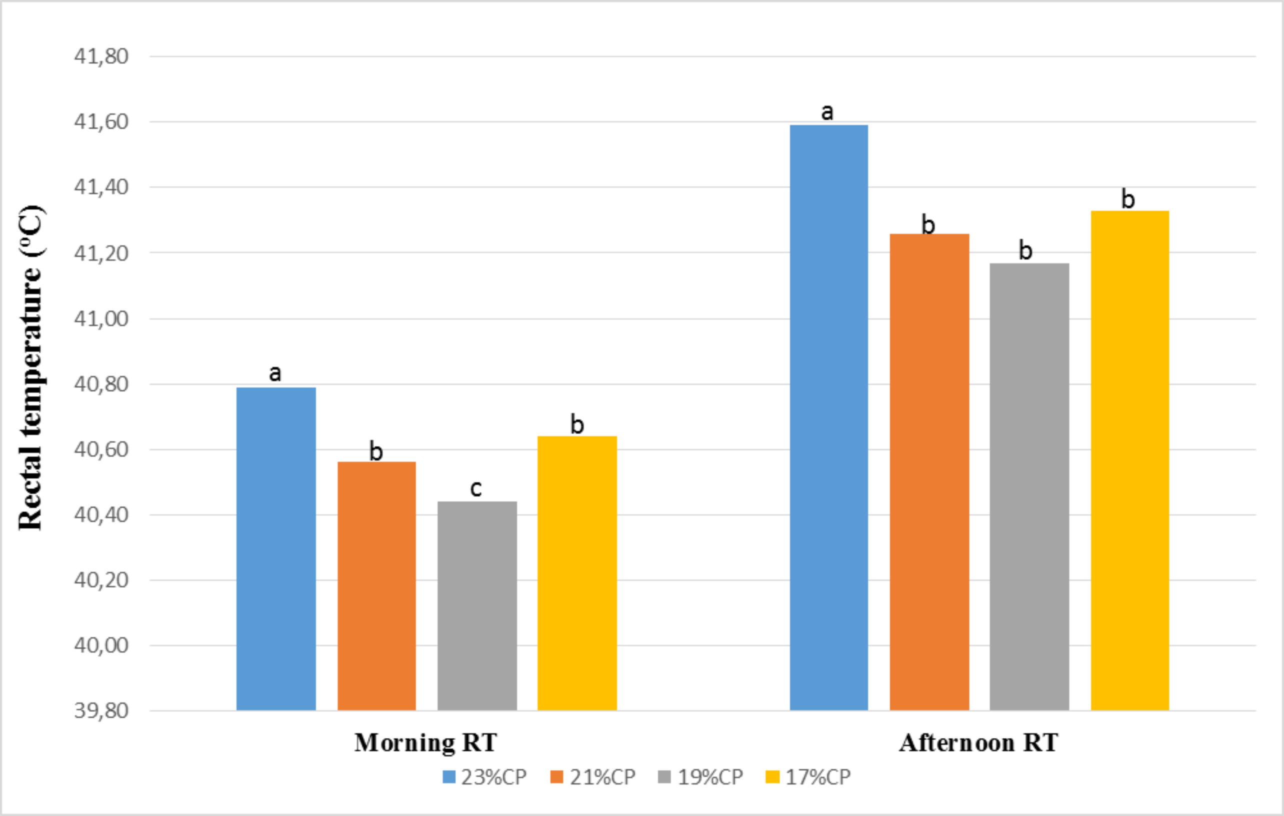

Fig. 1. Rectal temperature of broiler chicks fed varying dietary crude protein at optimum Dietary electrolyte balance

Bar means of treatments with different superscripts differed significantly ($P < 0.05$) using DMRT.

RT – Rectal temperature; TC – Temperature change; CP – Crude protein

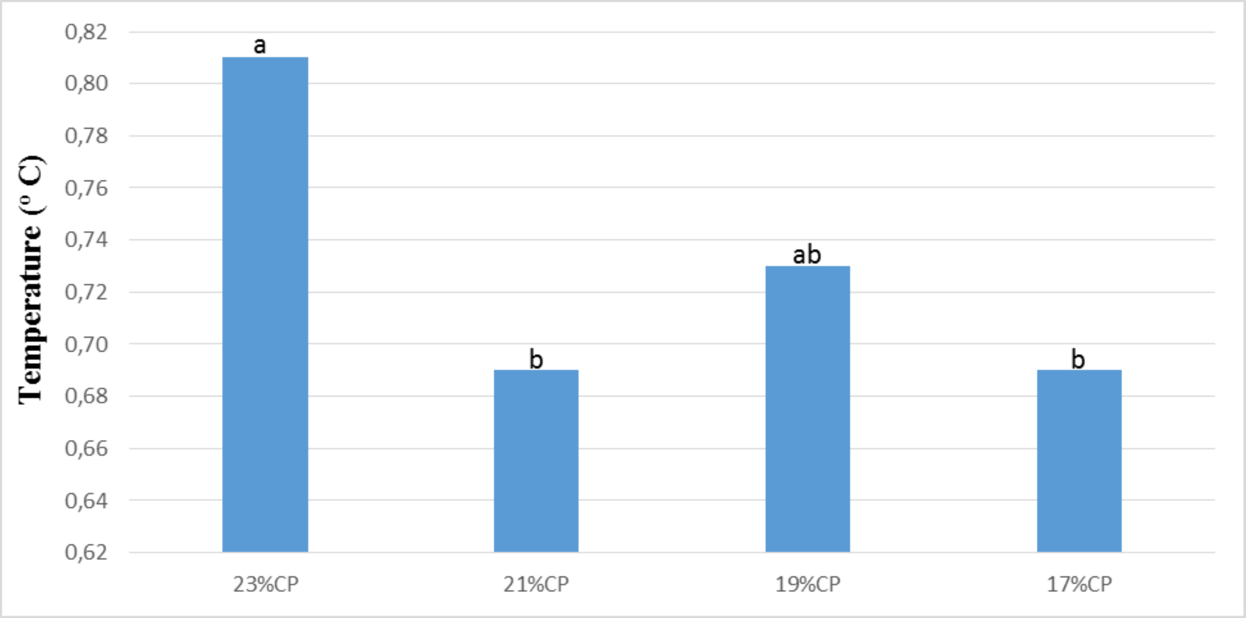

Fig. 2. Body temperature fluctuations in starter broiler chicks fed varying dietary crude protein Bar means of treatments with different superscripts differed significantly ($P < 0.05$) using DMRT CP – Crude protein.

Figure 3 revealed the relationship between varying dietary crude protein and protein efficiency ratio (PER) of heat-stressed broilers at pre-starter phase. A linear relationship existed, with decreasing PER observed with increasing levels of dietary crude protein. The R^2 value (0.86) indicated that about 86 % of the observed differences in PER of pre-starter chicks were as a result of dietary crude protein levels.

Figure 4 revealed the relationship between varying dietary crude protein and protein efficiency ratio (PER) of heat-stressed broilers at starter phase. A linear relationship existed, with increasing PER observed with increasing levels of dietary crude protein. The R^2 value (0.79) indicated that about 79 % of the observed differences in PER of heat-stressed starter chicks were as a result of dietary crude protein levels.

Figure 5 revealed the relationship between varying dietary crude protein and water intake of heat-stressed broilers at starter phase. A linear relationship existed, with increasing intake of water observed with increasing levels of dietary crude protein. The R^2 value (0.50) indicated that about 50 % of the observed differences in water intake of heat-stressed starter chicks were as a result of dietary crude protein levels.

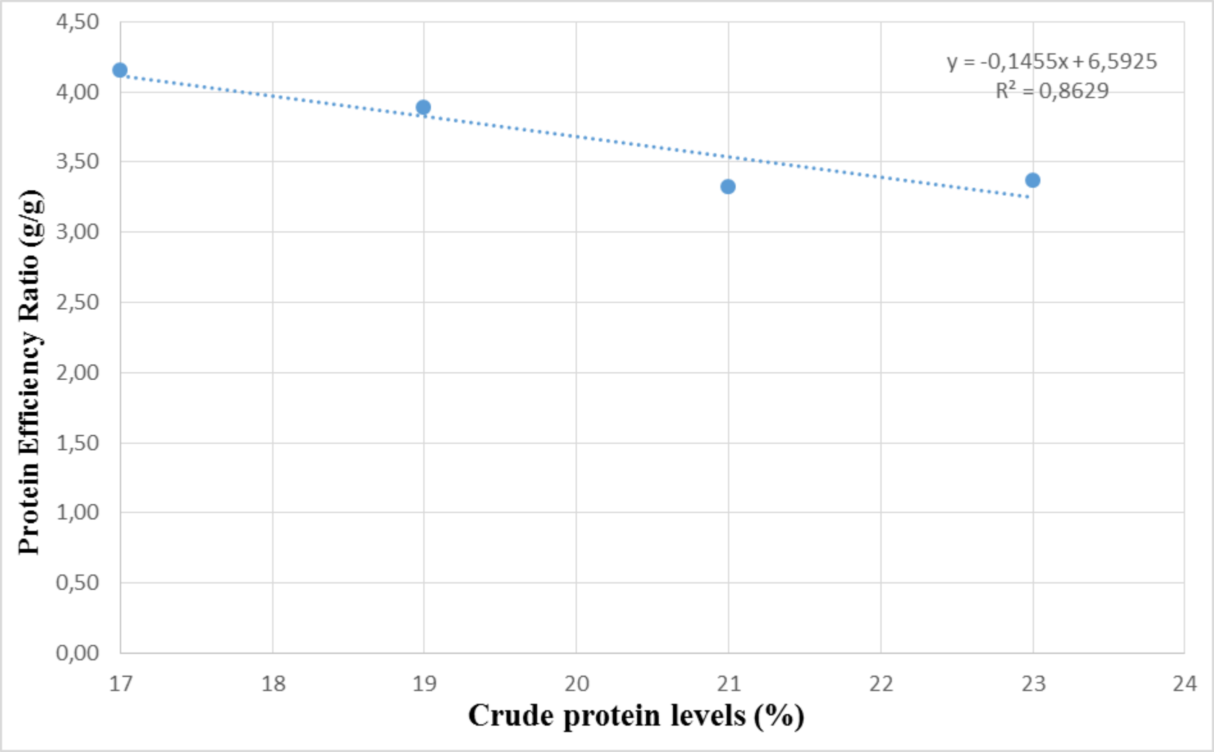

Fig. 3. Relationship between dietary crude protein levels and protein efficiency ratio in heat-stressed broiler chicks at pre-starter phase

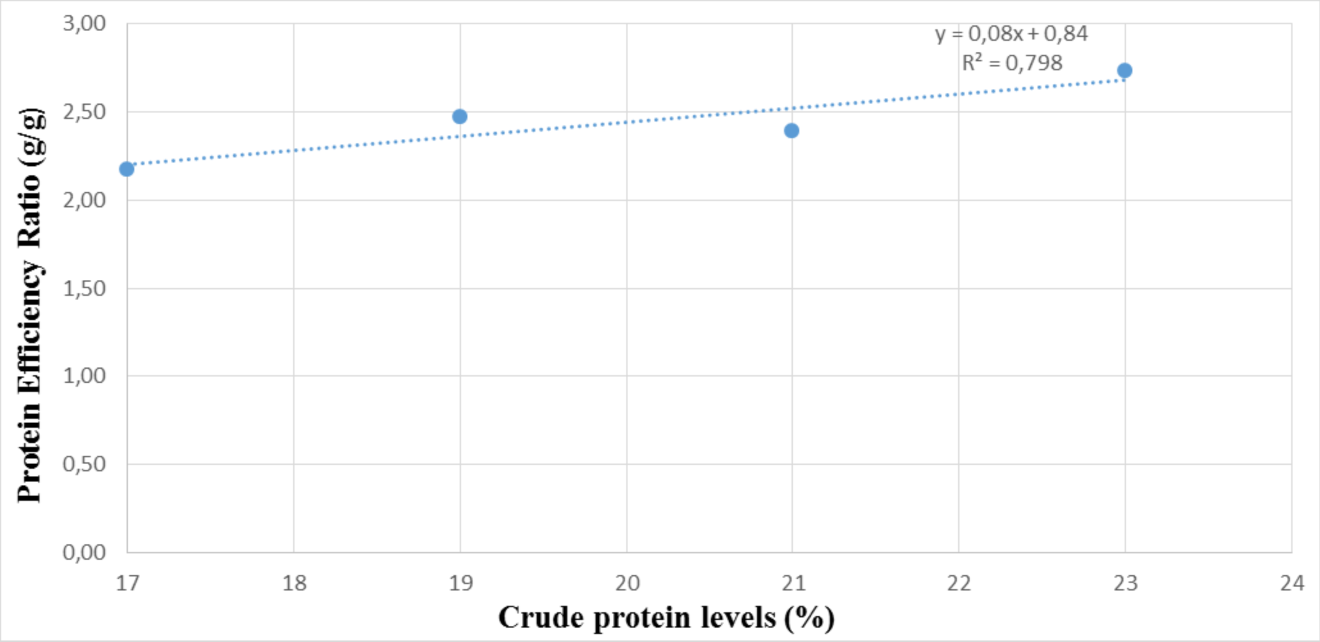

Fig. 4. Relationship between dietary crude protein levels and protein efficiency ratio in heat-stressed broiler chicks at starter phase

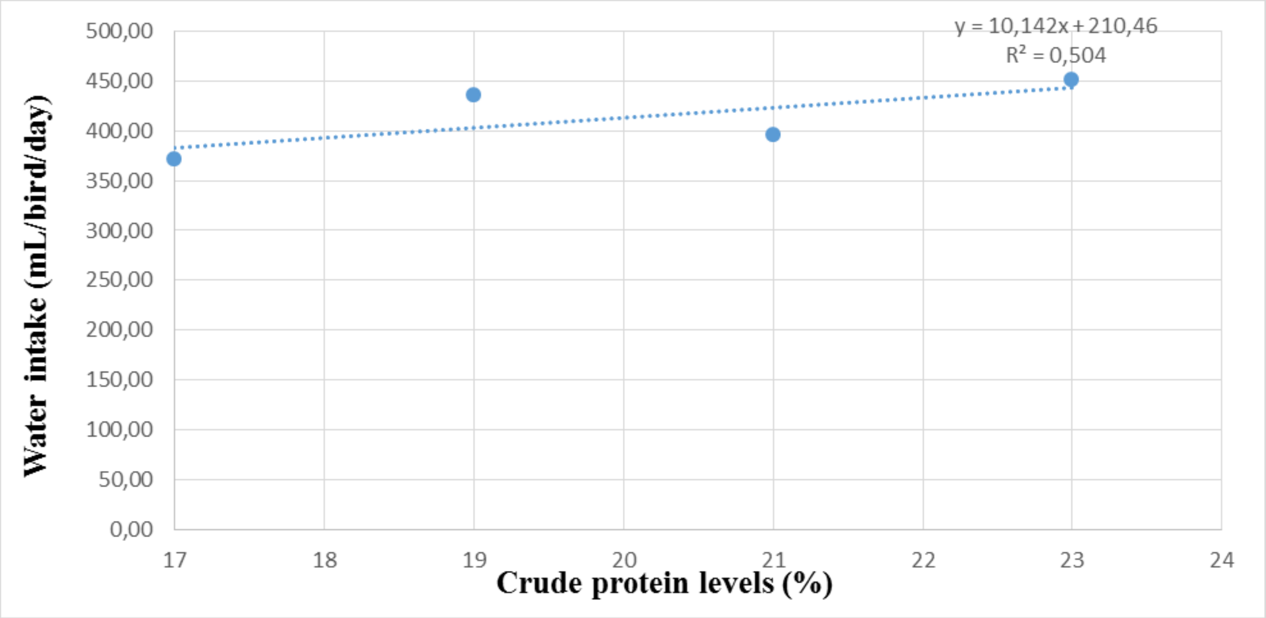

Fig. 5. Relationship between dietary crude protein levels and water intake in heat-stressed broiler chicks at starter phase

Tables 5 and 6 show the correlation between performance parameters and protein intake of heat-stressed broilers at pre-starter and starter phases, respectively. It was observed that a positive and significant ($P < 0.05$) correlation existed between feed intake, BWG, and protein intake at pre-starter phase. However, a non-significant positive correlation also existed among feed intake, feed efficiency, water intake, morning and afternoon RT. Also, a negative, non-significant relationship existed between feed intake and PER ($r = -0.91$) at pre-starter phase. Feed efficiency was positively correlated ($r = 0.97$; $P < 0.05$) with temperature change in pre-starter chicks. It was observed that no significant ($P > 0.05$) correlation existed between PER and other production parameters at pre-starter phase. Also, rectal temperatures in the morning and afternoon were not significantly ($P>0.05$) correlated with other production parameters at pre-starter phase.

At starter phase, a positive and significant relationship existed between feed intake and protein intake (r = 1.00; P < 0.01). However, a non-significant positive correlation existed between feed intake and BWG, feed efficiency, PER, water intake, rectal temperatures and temperature change. The BWG had a negative, but significant (P < 0.01) correlation with FCR. Feed efficiency was strongly correlated with BWG (r = 0.97; P < 0.05) and PER (r = 1.00; P < 0.01). No significant (P > 0.05) correlation existed between water intake and other production parameters.

Table 5. Correlation between performance parameters and protein intake of heat-stressed broiler chicks at pre-starter phase

Parameters	FI	BWG	FCR	Gain: feed	Protein intake	PER	Water intake	Morning RT	Afternoon RT
FI	1	0.95*	-0.77	0.73	0.98*	-0.91	0.55	0.25	0.40
BWG	0.95*	1	-0.93	0.91	0.95	-0.76	0.77	0.36	0.54
FCR	-0.77	-0.93	1	-0.99**	-0.76	0.46	-0.95	-0.33	-0.53
Gain: feed	0.73	0.91	-0.99**	1	0.74	-0.43	0.95	0.42	0.61
Protein intake	0.98*	0.95	-0.76	0.74	1	-0.93	0.52	0.43	0.57
PER	-0.91	-0.76	0.46	-0.43	-0.93	1	-0.17	-0.33	-0.41
Water intake	0.55	0.77	-0.95	0.95	0.52	-0.17	1	0.19	0.39
Morning RT	0.25	0.36	-0.33	0.42	0.43	-0.33	0.19	1	0.98*
Afternoon RT	0.40	0.54	-0.53	0.61	0.57	-0.41	0.39	0.98*	1
TC	0.67	0.86	-0.95	0.97*	0.73	-0.43	0.88	0.61	0.77

*P < 0.05; **P < 0.01, ns – not significant; FI – Feed intake, BWG-Body weight gain, FCR-Feed conversion ratio, PER – Protein efficiency ratio, RT – Rectal temperature, TC – Temperature change.

Table 6. Correlation between performance parameters and protein intake of heat-stressed broiler chicks at starter phase

Parameters	FI	BWG	FCR	Gain: feed	Protein intake	PER	Water intake	Morning RT	Afternoon RT	TC
FI	1	0.93	-0.87	0.81	1.00**	0.82	0.61	0.46	0.56	0.62
BWG	0.93	1	-0.99**	0.97*	0.93	0.97*	0.86	0.43	0.59	0.84
FCR	-0.87	-0.99**	1	-0.99**	-0.87	-0.99**	-0.92	-0.42	-0.59	-0.89
Gain: feed	0.81	0.97*	-0.99**	1	0.81	1.00**	0.95	0.41	0.59	0.92
Protein intake	1.00**	0.93	-0.87	0.81	1	0.82	0.61	0.46	0.56	0.62
PER	0.82	0.97*	-0.99**	1.00**	0.82	1	0.94	0.41	0.59	0.92
Water intake	0.61	0.86	-0.92	0.95	0.61	0.94	1	0.17	0.39	0.88
Morning RT	0.46	0.43	-0.42	0.41	0.46	0.41	0.17	1	0.98*	0.61
Afternoon RT	0.56	0.59	-0.59	0.59	0.56	0.59	0.39	0.98*	1	0.77
TC	0.62	0.84	-0.89	0.92	0.62	0.92	0.88	0.61	0.77	1

*P < 0.05; **P < 0.01, ns – not significant; FI – Feed intake, BWG – Body weight gain, FCR – Feed conversion ratio, PER – Protein efficiency ratio, RT – Rectal temperature, TC – Temperature change

4. Discussion

The results of present study were consistent with the observations of Macari et al. (2004), who noted that birds` adaptation to heat stress condition involves a deliberate reduction in feed intake in an attempt to lower endogenous heat production, provided that the physiological status of such organism cannot tolerate the excess heat load. Although, with ideal balance of dietary electrolytes, feed intake in heat-stressed birds on 23 % CP, 21 % CP and 19 % CP, did not differ in pre-starter chicks except for 17% CP, while no observable difference exited among dietary treatments at starter phase. The results of present study agree with the assertions of Oliveira et al. (2006), who affirmed that performance decline in broilers under heat stress conditions resulted from the inability of birds to expel excess endogenous heat load. Yahav and Hurwitz (1996) noted that broiler chicks exposed to high temperatures of 36ºC at pre-starter period maintained higher rate of viability when challenged with higher temperatures at finisher phase, and concluded that such adaptive responses were due to previous exposure to heat stress. Musharaf and Latshaw (1999) noted that the metabolism of crude protein ingested by livestock results in a greater increase in heat production than carbohydrates or fats. The results of present study partially contradicted the reports of Cheng et al. (1997) who noted that the provision of higher crude protein in diets of heat-stressed broilers, in attempt to compensate for low appetite, is detrimental to production parameters. The observed reason for such contradiction is that if the rations are well balanced in electrolytes, such assertion might not stand. However, contrasting reports were documented by Temim et al. (2000), affirming that low-CP diets are not beneficial to poultry under heat stress conditions. The results of present study have shown that low CP diets are indeed beneficial at optimum DEB of 270mEq/kg for starter chicks, but at a CP level not lower than 19 %, even though protein efficiency ratio in such low CP diets are much enhanced.

Awad et al. (2014) reported a decrease in performance of broilers fed diets with low CP, even when all essential amino acid requirements are met. However, from present findings, it can be hypothesized that extremely low CP diets are unbeneficial to broilers in a state of thermal comfort, but when heat-stressed, these low CP diets become beneficial in reducing endogenous heat contribution and increasing the efficiency of nutrients. The result of present study is consistent with the report of Si et al. (2004) who also noted reduced appetite in birds fed low-CP diets. The reason for this observation could have been as a result of the association between dietary CP levels, feed intake and protein intake, as a strong positive correlation existed between these parameters at pre-starter phase. The results of present study is consistent with the reports of Buyse et al. (1992) who noted reduced growth performance in response to increased heat production in male broiler chickens, while affirming that the heat increment in broilers fed a lower protein diet was as a result of elevated plasma triiodothyronine (T3) concentration, which may consequently increase heat production.

Soleimani et al. (2012) reported that performance of broiler chickens was negatively affected by dietary CP level reduction at both starter and finisher phases, regardless of feed-grade amino acid manipulation. Although, Aftab et al. (2006) established that the reduction of dietary CP will reduce both essential and non-essential amino acids, and could alter the balance of such in low-CP diets, with an aftermath on feed intake as the amount of ingested free amino acids into the blood stream increases in birds on low CP diets and may affect the balance of plasma amino acid profile. The results of present findings at starter phase partly corroborate the assertions of Bregendahl et al. (2002), indicating no feed intake differences in feeding low CP diets to broilers. A critical estimation of the feed intake trend in pre-starter and starter chicks on very low CP diet of 17 % in present study corroborate the report of Aletor et al. (2000) who observed higher feed intake in birds on low-CP diets. According to Waldroup (2007), reduced performance in broilers on low-CP diets could be associated with insufficient nitrogen for non-essential amino acid synthesis, because chickens on standard levels of dietary protein can synthesize the non-essential amino acids from excess essential amino acids, but when absent or in low supply, synthesis is reduced and performance decline is aggravated. Ali and Hossain (2010) stated that heat stress in broilers can be reduced by early-age thermal conditioning, as it is a sensitive process of induction of thermo-tolerant traits in immature neonatal chicks at an early age, by developing the hypothalamus, which is the thermo-regulatory centre, and has been proven to be the most appropriate age to exploit and induce thermotolerance in broiler chickens (Yahav et al., 2005). Present findings agree with the reports of Tan et al. (2010), who noted that heat stress during early post-hatch periods may result in greater adaptability to thermal stress even when endogenous heat production is higher. Since birds must maintain internal body temperature despite thermal oscillation, they do so at the expense of production, by diverting nutritionally beneficial molecules to homeostatic adjustments. Yahav and Mcmurtry (2001) reported that thermal conditioning retarded growth immediately, before a period of compensatory weight gain was attained and concluded that these thermal variables correlate with nutrient absorption. Early feed administration post-hatch coupled with pre-starter diet formulation also dictate thermal regulatory pattern in broilers. The results of current study corroborate the reports of Van Den Brand et al. (2010), who noted that chicks fed high density pre-starter diets had elevated body temperatures within the first three days post-hatch, and were more resistant to cold stress, because of the high energy density and metabolic heat production. Popoola et al. (2020d) noted that a degree centigrade fall or rise in body temperature of broiler chickens is sufficient to reduce performance, alter sound physiological state and divert nutritional metabolism in quest for ensuring thermal balance.

5. Conclusion

Starter chicks can well tolerate endogenous heat production without diverting nutrients needed for growth to regulation of homeostasis. However, they do so at the expense of increased water intake and drinking frequency, provided that an ideal balance of dietary electrolytes is provided in rations. Protein efficiency was decreased in pre-starter chicks with increasing dietary crude protein due to intolerance of excess endogenous heat load in the face of thermal oscillation.

Novelty and Importance of the research

The huge contribution of external factors, such as the earth`s surface temperature change, nutrition, and managerial strategies that determine the expression of useful genetic traits in fast-growing broiler chickens cannot be ignored and has necessitated adjustments in major contributing factors to beneficial traits expression in broilers such as nutrition. Crude protein ingestion cause a greater increase in heat production than carbohydrates or fat. However, controversy abounds on whether or not low CP diets are beneficial to poultry raised in hot environment. It is essential to investigate the effects of heat stress acclimation during early weeks on adaptability to high endogenous heat production in broiler chicks. Protein efficiency was decreased in pre-starter chicks with increasing dietary crude protein due to intolerance of excess endogenous heat load in the face of thermal oscillation, while starter chicks can well tolerate endogenous heat production without diverting nutrients needed for growth to regulation of homeostasis. However, they do so at the expense of increased water intake, provided that an ideal balance of dietary electrolytes is provided in rations.

Conflicts of Interest

The authors declare no conflicts of interest as regarding the publication of this paper.

Informed Consent

The authors confirm that written consent was obtained from all participants prior to the study.

Ethical Approval

The study received the ethical approval of the Institutional Animal Care and Use Committee, through the Agricultural Biochemistry and Nutrition Unit of the Department of Animal Science, University of Ibadan, Nigeria.

Contributorship

I. O. Popoola designed, implemented and analyzed data; O.R. Popoola and I.O. Popoola drafted the manuscript. I.F. Olaleru, I.O. Busari, F.J. Oluwadele, and O.O. Olajide contributed and approved the final draft of the manuscript.

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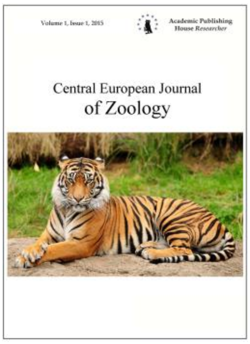

Spawning of Sole Fish and Its Larvae and Juvenile Rearing: A Review

Hamed H.E. Saleh [a], [*]

[a] Aquaculture Division, National Institute of Oceanography and Fisheries (NIOF), El-Fayoum, Egypt

Abstract

A number of scientific studies have investigated aspects of soles spawning and rearing of larvae and juvenile. Studies show that sole spawn readily in captivity, and the buoyant, fertilized eggs are easily collected. Stocking density during maturation should be 0.75-1.5 kg/m^2, and temperature should be kept above 16°C for *Solea senegalensis* or between 8 and 12°C for *Solea solea*. The onset of spawning is related to a rise in temperature occurring at above 10°C and viable eggs are found in the temperature range of 8-12 °C. Salinity should be kept constant around 33-35 ‰. Hormonal induction of ovulation for sole fish was successful with human chorionic gonadotropin (HCG), carp pituitary extract (CPE), luteinizing and releasing hormone analogue (LHRHa) and gonadotropin-releasing hormone agonist (GnRHa). Hatching of *Solea aegyptiaca* eggs occured after 48±3 h from fertilization at temperature 16-19 °C, while occured after 60-72 h at 14-16 °C, for wedge sole after 36-48 h at 19 °C and within 72-96 h at 11-17 °C for *Solea solea*. The growth rate for larvae has been increased gradually with increasing temperature until 20 °C then decreased. The best growth performance of larvae at photoperiods of 18 light: 6 dark and at salinity 20 ‰. Studies indicate that a mixture of inert and live food may increase the weaning success of sole fry, and this can be further enhanced by using attractants in the dry feed. Future experiments are needed to determine the ideal time to commence weaning and determine the minimum duration of this period. Studies on alternative feeding strategies are also required. Protein requirements for maximum growth of sole are ranges between 50 % and 65 % of diet. This review clearly indicates, that albeit there exists many unsolved problems, sole remains a very attractive candidate for marine aquaculture and has a very big potential for future farming.

Keywords: soles spawning, rearing of larvae and juvenile, stocking density, temperature, salinity, photoperiods, hormonal induction, protein requirements.

1. Introduction

Senegal sole (*Solea senegalensis*) is currently an important focus for research in aquaculture for flatfish in Europe especially in Portugal and Spain. However, before a reliable mass production technology can be transferred of Senegal sole to an industry, several aspects of its culture still need to be resolved and improved (Agulleiro et al., 2006). The culture technology for Senegal sole (*Solea senegalensis*) is mainly impaired by the lack of methods to control reproduction in captivity (Cabral, 2000). This pattern of spawning is also observed in wild Senegal sole in captivity under natural temperature regimes (Anguis, Canarate, 2005). However, the natural spawning of wild

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[*] Corresponding author
E-mail addresses: hhsaleh90@gmail.com (H.H.E. Saleh)

caught fish requires long periods of adaptation to captive conditions, and when this occur, it is often unpredictable and the quality of the egg produced is variable.

Solea senegalensis is a species of high commercial value and is a potential candidate for commercial scale aquaculture. It easily adapts to highly saline environments. Senegal sole is a species traditionally exploited in aquaculture. But, there are some problems with sole growth in relation to nutrition in intensive and semi-intensive systems (Dinis, 1992; Dinis et al., 1999; Imsland et al., 2003).

Imsland et al. (2003) published a review of the culture potential of *Solea senegalensis* compared with *Solea solea*. More information is available on the commercial husbandry of *Solea senegalensis* (Dinis et al., 1999; Imsland et al., 2003), but this flatfish offer important differences in growth performance and optimal thermal regime.

The common sole is a respectable and highly-priced fish for consumption with a relatively fast production cycle ranging from 1-1.5 years at 20°C (Howell, 1997; Imsland et al., 2003). Moreover, sole larvae are regarded fairly easy to rear (Howell, 1997), making it a prime candidate for the expansion intensive recirculation fish farms in northern Europe. However, the mortality of larvae and juveniles are still high, compromising the intensive production of this species.

Successful culture of marine fish larvae depends also of many biological and physical aspects of the rearing systems. It is important that the rearing systems used to raise larvae of newly cultured species should be designed to avoid confounding variables. Most of the marine fish larvae are visual feeders and are dependent on a certain level of light to identify and attack the preys. As a result, feeding success depends not only on the quantity of suitable food but also on the visibility of the preys in the rearing tanks (Ina et al., 1979; Hunter, 1980).

According to Segner et al. (1993, 1994) the knowledge of the physiology of nutrition during on-growing provides necessary support for the understanding of larval nutritional needs. Moreover, present efforts to replace live food by inert micro-diets depend largely on such knowledge (Bengtson et al., 1993; Yufera et al., 1996). Indeed, *S. senegalensis* exhibited good growth during the larval period when fed live prey (Vazquez et al., 1994; Dinis, Reis, 1995a), the weaning (transition from live prey to compound diet feeding) induces poor growth and mortality (Dinis, 1992; Marin-Magan et al., 1995).

The objective of this review is to provide information on spawning of sole fish and its larvae and juvenile rearing, as this may assist scientists in pinpointing future challenges that need to be solved if this species is to become an important aquaculture species.

2. Results

Common sole

Habitat and biology

The sole usually lives on sandy and muddy seabeds in depths between 10 to 60 m. They mainly hunt for food at night and feed on worms, mollusks and small fishes and crustaceans. During the day they lie on the seabed buried in sand with only the eyes showing (Picton, Morrow, 2010). Adults occur at a temperature range of 8.0-24.0 °C (Moreira et al., 1992). Usually solitary. Waden sea is the most important nursery area (Murua, Saborido-Rey, 2003). Frequently found pelagically during spawning migrations (Muus, Nielsen, 1999).

Geographical distribution

astern Atlantic: southward from Trondheim Fjord (including North Sea and western Baltic) and Mediterranean Sea (including Sea of Marmara, Bosporus and southwestern Black Sea). Elsewhere, southward to Senegal, including Cape Verde (Desoutter, 1990).

Description

This is a right-eyed flatfish which has a very distinctive shaped head with a rounded snout and a small, down-turned mouth. There is a row of short, fine filaments surrounding the head and mouth. The dorsal fin begins in front of the eyes and runs the length of the body where it joins with the tail and anal fins. The edges of the dorsal and pelvic fins are usually white. The background colour is greyish-brown with darker blotches, the pectoral fin has a distinctive black tip. Adult fish are usually 30-40 cm long, but large individuals may grow to 60 cm (Picton, Morrow, 2010).

Taxonomy
Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Superclass: Pisces
Class: Osteochthyes
Subclass: Actinopterygii
Order: Heterostomata
Suborder: Pleuronectiformes
Family: Soleidae
Genus: *Solea*
Species: *solea* (Linnaeus, 1758);
aegyptiaca (Chabanaud, 1927);
senegalensis (Kaup, 1858)

Local names
Arabic: Mousa (Egypt), Sole (Tunisia), Mousa (Lebanon)
English: Common sole, Dover sole, Sole
English: Egyptian sole for *Solea aegyptiaca*
English: Senegal sole for *Solea senegalensis*
French: Sole, Sole commune
German: Seezunge, Zunge
Spanish: Lenguado
Portuguese: Linguado
Danish: Tunge
Dutch: Tong, Zeetong
Russian: Morskoi yazyk

Spawning of sole

Broodstock

Sole readily spawn naturally in captivity and the buoyant, fertilized eggs are collected easily from the spawning ponds. This procedure is simple and effective and consequently, most published accounts of sole spawning in captivity are descriptive rather than analytical. The review article of Baynes et al. (1993) review the conditions and procedures used to obtain natural spawning from captive broodstock. Also, this review describes the characteristics of egg production. It is clear that spawning of the species has been obtained under a wide range of conditions. The time of year may differ, even at the same location, but analysis of published data shows that the temperature at which it commenced was related directly to the minimum temperature of the previous winter. It has been shown that annual relative fecundity varies from 10 to 140 eggs per gram of female weight with a tendency for higher values to have occurred following winter temperatures of less than approximately 12 °C. Variable fertilization rates are a common feature of eggs spawned by captive stocks with average fertilization rates for the complete spawning seasons ranging from 20 to 80 % (Howell, 1997).

Spawning time

Spawning of sole has been displaced by several months by manipulation of temperature and photoperiod (Devauchelle et al., 1987) and the temperature alone (Lenzi, Salvatori, 1989). In the wild, spawning is associated with temperature (Brittany, December/ January, Deniel, 1990; North Sea, early spring, Fonds, 1976) and viable eggs are found at a temperature between of 8-12 °C. In captivity with ambient conditions Devauchelle et al. (1987) conditions for spawning and eggs production were reported from 229 batches of sole in the period 1974–1986. These fish were wild caught and divided into two groups. One group of spawners was submitted to natural variations in temperature and photoperiod, whereas the other group was maintained under artificial cycles (temperature and light advanced by 4 months). The spawning was carried out in the ambient group between March and mid-May with temperatures between 8 and 12 °C and day length of 11-16 h of light. The corresponding dates for the advanced group were September to mid-November with temperatures between 8 and 15 °C with day lengths of 11-16 h of light. In the advanced group fewer eggs and more variable viability rates were observed. The researchers concluded that the sole has an asomewhat narrow temperature range for spawning (8 and 12 °C) and that the species responds to photoperiodic and thermal stimulation in similar ways to those of most fish species in temperate areas.

Previous studies about the reproduction of soleid species in Egypt. El-Husseiny (2001) reported that the female Gonado-somatic index (GSI) of *Solea aegyptiaca* in Quarun Lake, increased progressively to reach its maximum value in January, while the minimum value was recorded in July, whilst Ahmed et al. (2010) found that monthly changes in GSI for females and males *Solea aegyptiaca* showed a specific breeding season which extends from January to June. Both sexes reached the highest GSI values in January, while the lowest values were in June and August for females and males, respectively. Salman (2014) confirmed that the common sole (*Solea solea*) in Bardawil Lake is a winter spawner and spawned once a year. Mehanna (2014) confirmed that the *Solea solea* in Bardawil Lake is a winter spawner and spawn once a year. And the spawning period occurs between October and April with a peak activity during December. In the wild, common sole (*Solea vulgaris*) are reproductively active throughout the period from December to late March (Assem, 1995).

Reproductive strategies

Baynes et al. (1994) published a description of spawning behaviour of *S. solea* in captivity (tanks were 1 m deep × 3 m in diameter). Video recordings of the fish showed that the fish swim in pairs, with the male swimming into a position under the female where the genital pores are very close. This position is necessary, as the sperm duct opens at the end of a papilla on the dorsal surface and the female's cloaca opens on the ventral margin. They would only be adjacent when the male is underneath the female. The male and female then swim together towards the water surface, with closely synchronized body movements, remaining together for up to 70 s, while maintaining a head-up position just beneath the surface.

To summarize the environmental conditions used for broodfish we find in published studies: sex ratio of 0.5-3 males to each female, moderate densities (0.6-3.0 kg/m^3, Devauchelle et al., 1987), large tanks (>10 m^3), with light intensity of 20-1500 lux, temperature should be kept between 8 and 12 °C with ambient fluctuations. Saleh et al. (2016) reported that, three different broodstock sex ratios: 1:1, 1:2 and 2:1 (male ♂: female ♀) and four different stocking densities: 0.5, 0.75, 1, 1.25 kg/m^3 were tested for *Solea aegyptiaca*. Larvae production of *S. aegyptiaca* was affected by different broodstock sex ratios and stocking densities and improved at a sex ratio (1♂:2♀) and the best stocking density was 0.75 kg/m^3. Broodfish are fed *ad libitum* with fresh mollusc and polychetes at an average rate of about 10% body weight per week (Baynes et al., 1993), *Solea senegalensis* broodstock were fed five days a week, three days with mussels and two days with small cuttlefish; one of the days on which they were fed with mussels, frozen polychaetes were added (Blanco-Vives et al., 2010) and *Solea vulgaris* broodstock were fed warms and shrimps, with daily rate of 1.5 % of the body weight (Assem et al., 2012). The fish are allowed to spawn naturally and fertilized eggs are collected in the water column. Temperatures are allowed to follow the annual cyclic regime were the difference between maximum and minimum temperature should be around or less than 12 °C. The first signs of spawning are associated with raising temperature in spring and this temperature rise may be an important environmental determinant for spawning. Fecundity varies between 10 and 140 eggs/g and fertilization rate between 20 and 80% (Baynes et al., 1993). Bedoui (1995) *Solea senegalensis* was reared on experimental scale. The rearing was conducted from broodstock to juveniles of 2 months. The broodstock were collected from the wild and acclimatized in a raceway with a sand bottom. Natural spawning was obtained at 18 °C during a 3-month period (April-June). The pelagic egg size ranged from 0.99 to 1.02 mm. The incubation was carried out in stagnant water lasting 42 h at 19 °C.

Dinis et al. (1999) reported that culture of *Solea senegalensis* in Portugal and Spain in the period 1993–1997. Natural spawning of broodstock in captivity, was the only way to obtain viable eggs. The broodstock feeding regime was based on squid, *Loligo vulgaris*, and was supplemented with polychaetes, *Hediste diversicolor*, during the final maturation. The temperature played a very important role in the beginning and duration of the spawning period, with egg emission stopping below 16 °C. Observed duration of the spawning period ranged from 4 to 6 months. Stocking density in the maturation tanks should be 1-1.5 kg/m^2. Dinis et al. (2003) indicate that broodstock density might be up to 5 kg/m^2. The weight of eggs collected daily during the spawning season ranged from 0 to 180 grams for a broodstock of 15 fish. The fertilization rates for eggs ranged between 20 % and 100 %, and the proportion of viable eggs (proportion of fertilized eggs hatching) was 72.1 ± 26.5 %. Differences in egg size between batches were revealed, with egg size tending to decrease during the spawning season. Similar trend is found for *S. solea* (Baynes et al., 1993). In many fish species the percentage of viable eggs is related to age (Marteinsdottir, Begg, 2002).

Dinis and Reis (1995b) studied the broodstock management and larval rearing of *Solea senegalensis* and concluded that after 7 months in captivity a wild broodstock spawned naturally at temperature ranging from 16.5±0.5 °C to 22±1.0 °C and salinity from 30 to 35 ‰. Egg with 100 % fertilization presented viability ranging from 100 % to 90 %.

The fertilization rate of Wedge sole (46.8-73.8 %) was discussed by Herrera et al. (2008), who concluded that the fertilization rates are slightly higher than those found for *Solea senegalensis* (39.8-67.6 %) by Anguis and Canavate (2005), and other flatfish species new for aquaculture as *Colistium nudipinnis* and *Colistium guntheri* by (Tait, Hichman, 2001).

Saleh et al. (2016) reported that, the percent of hatching rate varied between 90 and 95 % at water temperature of 13-19 °C and salinity 31-34 ‰. Similar results by Salas-Leiton et al. (2012) reported that, hatching percentage is ranging between 80.7 and 95.7 % at water temperature of 20 °C and salinity 18-33 ‰ for *Solea senegalensis*. Moreover, Assem et al. (2012) observed that, hatching percentage is ranging between 89 and 90 % at water temperature of 17±2 °C and salinity 34±1 ‰ for *Solea vulgaris*. While Blanco-Vives et al. (2010) reported that, hatching percentage was 78.1 % at water temperature of 16-19 °C for *Solea senegalensis*. Dinis et al. (1999) mentioned that, hatching percentage was 72.1 ± 26.5 % for *Solea senegalensis*. Moreover, a hatching rate of wedge sole (58.3-85.2 %) was recorded by Herrera et al. (2008). As well as described by Anguis and Canavate (2005) for Senegal sole (55.4-70.9 %) and other cultured flatfish species as halibut (14-51%) by Mazorra et al. (2003).

Induced spawning

Hormonal stimulation is usually done with spawning of fish which are not normally spawned in captivity. For fish species which naturally spawn under confinement, hormonal manipulation is done to synchronize spawnings of many females for mass production of fry. Human chorionic gonadotropin (HCG) has been used successfully to induce ovulation and spawning in a number of fish species. It is used either alone or in combination with other hormones. One of the advantages of using HCG over other hormones is that its potency can be standardized in international units and results can be compared between users (Lee et al., 1988).

Ramos (1986a) HCG can be used to induce spawning in the common sole. The spawning was done by injecting HCG intramuscularly in single doses ranging from 250 to 1000 IU/kg fish. Low doses of HCG (250-500 IU/kg fish), injected into females with oocytes in the final stages of vitellogenesis induced spawning with the highest fertilization rate, number of eggs and number of spawnings. Saleh et al. (2016) reported that, artificial spawning of *Solea aegyptiaca* were achieved using HCG (total dose 7000 IU/kg fish body weight). Sharaf et al. (2017) reported that, artificial spawning of *Solea aegyptiaca* and *Solea vulgaris* were achieved using pituitary gland extract for carp fish (total dose 4 mg/kg of fish body weight).

In the other studies, Assem et al. (2012) artificial spawning of *Solea vulgaris* were achieved using carp pituitary extract (CPE) from 40-70 µg/fish (equal to 200 µg/ kg fish) or HCG from 2300 to 3000 IU/fish (equal to 10000 IU/ kg fish) as a priming dose, followed by luteinizing and releasing hormone analogue (LHRHa) from 52-60 µg/fish (equal to 200 µg/kg fish) in the resolving dose. The total number of fertile eggs ranged from 87000 to 120000 eggs/ spawn which represented 430±108 eggs/gram of fish body weight. Fertilization rate ranged between 82-88 % and the hatching rate was about 90 % at 15±2°C and water salinity of 34 ‰.

Ramos (1986b) luteinizing hormone-releasing hormone analogue (LH-RHa) can be used to induce precocious ovulation in *Solea solea*. The spawning was done by injecting LH-RHa at doses of 10 µg/kg of body weight. The results suggest that LH-RHa accelerated maturation of the ovaries in females.

Agulleiro et al. (2006) induction spawning of Senegal sole (*Solea senegalensis*) using different methods for gonadotropin-releasing hormone agonist (GnRHa) and concluded that: females injected with a dose of 5 µg GnRHa/ kg three times a week, or treated with a single GnRHa loaded implant (50 µg/kg) showed multiple ovulations and spawns within a period of approximatiple 30 days. Mylonas and Zohar (2001) listed nine species of flatfish where ovulation and/or spawning were stimulated using a different GnRHa delivery system ranging from 30 to 1000 µg/kg.

Assem (1995) reported that, in artificial spawning of *Solea solea,* the total number of spawned egg varied between 1.3x 10^6 eggs per kg minimally and 2.1x 10^6 egg per kg maximally at water temperature 17±2.2°C and salinity 34±1‰. Herrera et al. (2008) indicated that for Wedge sole (*Dicologoglossa cuneata*) a relative fecundity reached 1.06-2.33x 10^6 egg kg per spawning season, gametes are released in a wide temperature range, 10-21 °C. From these finding, it is concluded that hormonal induction of ovulation for sole fish was successful with HCG, CPE, LHRHa and GnRHa.

Live food of sole larvae

In the hatchery production of aquatic animals for aquaculture, live foods such as microalgae, rotifer (*Brachionus plicatilis*), and brine shrimp (*Artemia* sp.) have been used throughout the world.

Microalgae

Microalgae are used to directly feed the rotifer or to indirectly feed the larvae at the first step of feeding. Algae helps to hold the quality of water parameters at optimum ranges in larvae tanks, stimulates larvae immune systems, minimises bacteriological contamination and nitrogen concentration (Ucal, 2002).

In Mediterranean country hatcheries, reserving of algae is made on wider areas than needed by production, by taking stock culture as base. Generally chosen species: *Isochrysis galbana, Nannochloropsis oculata, Nannochloropsis gadinota, Tetracelmis suecica*. Even though some of these species are not enough nutrition especially on ω3 fatty acids (HUFA) concentrations, they are chosen because of their rich protein potentials. Sizes, production aptitudes and inexistence of side effects like toxicity, are also matters for the selection of these species (Ucal, 2002).

Parameters affecting algal growth and production rates are; temperature: 18-24°C, salinity: 20-35 ‰, lighting force: 1,000-2,000 luxes on small scale culture, 10,000 luxes with mass culture, pH: 7.5-8.5 (Ucal, 2002).

Rotifers, *Brachionus plicatilis*

Rotifers are a valuable living food for the aquaculture of fish larvae and crustaceans (Lubzens et al., 1989). Specifically, *Brachionus plicatilis* is routinely used in marine aquaculture, being fed to more than 60 marine finfish and 18 species of crustaceans (James et al., 1983; Lubzens et al., 1997; Abu-Rezq et al., 2002). Many species of algae can be used for growing rotifers, the choice being largely dependant on what is available, what the culturist has worked with before, ease of culture under local conditions, exact nutritional requirements for rotifers and larvae feeding on the rotifers. When choosing the algae species, one must consider both the requirements of the rotifers as well as the needs of fish larvae.

The rotifers feeding by filtering bacteria, algae, protozoa, etc. Different species are present. Small type is about 100-200 µ, Large type is about 130-340 µ in lorica lenght. Usually bakers yeast is used for preliminary culture, but in some cases, some special nutrients (rich in HUFA and vitamins) are used for enrichment. Optimum culture conditions for rotifor culture: Temperature: 25-27°C, pH: 7.5-8.5, salinity: 25 ‰, light: 2000 lux., NH_3: <1 ppm, oxygen: 5-7 ppm (Ucal, 2002).

Artemia

Feeding *Artemia* cysts for young fish larvae is still necessary in commercial hatchery operations. The nutritional quality of commercially available *Artemia* strains is relatively poor in docosahexaenoic acid (DHA, 22: 6n-3) and eicosapentenoic acid (EPA, 20:5n-3), two major determinants of fish food value and also the price of *Artemia* cysts. It is a common practice to enrich these *Artemia* cysts with marine oils emulsions (Hanaee et al., 2005).

The genus *Artemia* comprises several species and strains, and differs in biometrical characteristics (nauplii size and cysts diameter), and in biochemical composition (fatty acids, vitamin C and carotenoid level) (Van Stappen, 1996). Regarding their content of n-3 HUFA, freshly-hatched nauplii of all species examined so far appear to be very low in DHA, whereas the amount of EPA appears to be species-specific (Navarro et al., 1993; Triantaphyllidis et al., 1995; Han et al., 1999).

Artemia nauplii, as passive filter feeders, are unable to select the food particles, and their ingestion therefore depends on the size and the availability of the particles in water column.

Optimal sizes of food efficient ingestion are known to be stage, and species-specific and range between 5 and 100 µm for filter feeders (Jones, Gabbot, 1976).

Nowadays, many different enrichment emulsions have been formulated into the fatty acid composition of their triglycerides. In this regard, the traditional formulations rich in EPA have been replaced by new products rich in DHA and arachidonic acid. To reduce the risks of oxidation for these fatty acids, higher concentrations of vitamin E are incorporated into the emulsions. Also, vitamin C has been incorporated in booster formulation of the enrichment diets offer more possibilities to cover the needs of different species and help to reduced problems related to stress resistance, diseases, malformation, and pigmentation in many fish species (Sorgeloos et al., 2001).

Several factors are critical for hatching the large quantities needed in condition of fish larval. These include cyst disinfection or decapsulation prior to incubation, and hatching under the following optimal conditions: constant temperature of 25-28°C, minimum pH of 8, 15-35 ‰ salinity, near saturated oxygen levels, maximum cysts densities of 2 g/L, and strong illumination of 2000 lux (Lavens, Sorgeloos, 1996). All these factors will affect the hatching rate and maximum output, and hence, the production cost of the harvested *Artemia* nauplii (Sorgeloos et al., 2001).

The larvae's oral cavity enlarges during growing. For this reason Rotifer is replaced with *Artemia* which is bigger size. 10-15 days after hatching, larvae are fed by *Artemia* of size 430 µ. After this period, specially enriched *Artemia* of 500 µ in total length are used. Because of its floating capabilities and increased feeding potential, *Artemia* is a good prey for fry. Mostly important characteristic of *Artemia* is its cyst production (which holds the embryo during its resting phase). Cyst incubation endures 24 hours in salt water at 30°C under a highly lighted and ventilated area. Additional 12-24 hrs is required for enrichment of metanauplius (Ucal, 2002).

Importance of live feeds in larval feeding

Live feeds (as Rotifers and *Artemia*) are able to swim in the water column and are thus constantly available to the larvae. Formulated diets tend to aggregate on the water surface or, more commonly, sink quickly to the bottom, and are thus normally less available to the larvae than are the live feeds. In addition, the movement of live feed in the water is likely to stimulate larval feeding responses, since evolutionary history has probably adapted them to attack moving prey in nature. Formulated diets are generally capable of moving only in a downward direction, towards the bottom. Finally, live prey, with a thin exoskeleton and high water content, may be more palatable to the larvae once taken into the mouth, compared to the hard, dry formulated diets. Any food must enter the mouth whole (i.e. the larva's mouth gape must be of sufficient size for particle ingestion to occur) and they are quickly either accepted or rejected on the basis of palatability (Aljilany et al., 2016a).

Larvae feeding regime on live food

Solea senegalensis larvae were fed on day 3 using Rotifer as first prey followed by *Artemia* nauplii (Dinis, Reis, 1995b). *Solea solea* larvae are reared from the fertilized egg until 28 days after hatching (DAH), with a feeding regime based on live food only. Larva's were fed on Rotifer 25-35 individuals (ind.) ind./ml from 4 DAH until 10 DAH and 35-25 ind./ml from 11 DAH until 15 DAH. Larva's were fed *Artemia* nauplii 1-4 ind./ml from 10 DAH until 17 DAH and 4-2 ind./ml from 18 DAH until 22 DAH. Larva's were fed on *Artemia* metanauplii 1-6 ind./ml from 12 DAH until 21 DAH and 6-12 ind./ml from 22 DAH until 28 DAH. Microalgae (300000 cell/ml) was added to the tanks from 4 to 15 DAH (Palazzi et al., 2006).

Solea senegalensis larvae feeding regime, Rotifers from 3 to 7 DAH, and *Artemia* metanauplii from 8 to 20 DAH were supplied. Rotifer's density was adjusted twice daily (08:00 and 15:00) to 10 rotifers/ml at the beginning of the feeding period. This density was gradually increased to 20 rotifers/ml for 7 days-old larvae. At 8 DAH 2 *Artemia*/ml was provided and rotifer supply ceased. *Artemia* density was also adjusted twice daily and increased to 3 and 4 metanauplii/ml at 10 DAH, and from 11 to 15 DAH, respectively. From 16 DAH onwards 7 *Artemia*/ ml were given. Live prey was not allowed to fall below 40 % of setup values (Canavate et al., 2006).

Solea senegalensis larvae feeding regime, rotifers (*Brachionus picatilis*) were added to tanks daily at a density of 20 ind./ml from 3 to 7 DAH. *Artemia* nauplii at a density of 2-3 nauplii/ml/day were introduced from 8 to 30 DAH. Three to five metanauplii/ml/day were added from 27 to

30 DAH. Green algae (*Nannochloropsis* sp.) was added to the tanks in a concentration of 300,000 cells/ml/day from day 3 to day 7 DAH (Blanco-Vives et al., 2010).

Solea senegalensis larvae feeding regime, the feeding consisted of 15 rotifers/ ml from 2 to 4 DAH, and 20 rotifers/ml from 5 to 8 DAH, and 10 *Artemia* metanauplii/ ml from 9 to 13 DAH, and 15 metanauplii/ml from 14 to 17 DAH and 20 metanauplii/ml from 18 to 21 DAH. *Nannochloropsis gaditana* (1 mg/L) was added to the tanks from 2 to 8 DAH to enhance background and rotifer nutritional status (Salas-Leiton et al., 2012).

Solea vulgaris larvae feeding system from first feeding to 28 DAH were based on live food only. Larva's were fed on rotifer (10 ind./ml) from 3 DAH until 12±2 DAH. From 14±1 DAH, larvae fed *Artemia* nauplii (3-5 ind./ml). At the age 25 DAH, it fed on *Artemia* metanauplii 8-12 ind./ml (Assem et al., 2012; El-Dahhar et al., 2013).

Solea aegyptiaca larvae were rearing from 4 DAH until metamorphosis stage, a feeding system based on live food only. Larva's were fed rotifer (20 ind./ml) from 3 DAH until 15 DAH. From 8 DAH, larvae fed *Artemia* nauplii (10 ind./ml). *Artemia* nauplii were introduced at 8 DAH and their density was gradually increased, becoming the only prey offered from 16 DAH. Feed was offered by hand at three meals/day (9:00, 13:00 and 16:00 h). Microalgae (*Nannochloropsis oculata*) at a final concentration of 5x105 cells/ml were also added to the rearing tanks from first feeding. By the age of metamorphosis stage, it fed *Artemia* metanauplii (8-12 ind./ml) (Aljilany et al., 2016a).

Using *Artemia* of sole feeding

Typically sole larvae and post-larvae are fed 2 to 4 times daily (Conceicao et al., 2007), but many studies are based on a feeding regime consisting of 2 meals per day (e.g., Engrola et al., 2007; Villalta et al., 2008). Ronnestad and Conceicao (2012) mention that sole are likely able to process a new meal every 2 hours, advising a high feeding frequency for this species. This suggests that commonly used feeding frequencies of 3-4 meals per day may not be sufficient to realize the maximum growth potential of Senegalese sole. It should be noted that even if high concentrations of *Artemia* are maintained in the larval tanks, these prey organisms loose quickly nutritional value, e.g., protein and essential fatty acids due to *Artemia's* own metabolism. The importance of optimal nutrition on different aspects of fish larvae quality such as skeletal deformities (Cahu et al., 2003), metamorphosis success (Harboe et al., 2009) and digestive performance (Ronnestad et al., 2007; Engrola et al., 2010) has been well demonstrated. Proximate analysis (% of dry weight in *Artemia*) and fatty acid composition (% of total fatty acids methyl esters) of the *Artemia* are shown in (Table 1) (Bonaldo et al., 2011).

Table 1. Chemical composition (% of dry weight) and fatty acids composition (% of total fatty acids methyl esters) of *Artemia* (Bonaldo et al., 2011).

Chemical composition					
Crude protein	55.3	Crude fat	15.4	Ash	13.0
Fatty acid composition					
14:0	0.6	15:0	1.2	16:0	11.1
18:0	5.1	Total saturated	18.0	16:1n-7	2.3
18:1n-9	20.2	18:1n-7	6.5	20:1n-9	0.6
20:1n-7	0.1	Total monounsaturated		29.7	
18:2n-6	5.8	20:2n-6	0.3	20:4n-6	0.8
Total n-6 PUFA	6.8	18:3n-3	29.5	18:4n-3	4.8
20:5n-3	1.1	Total n-3 PUFA	35.4	n-6/n-3	0.2

Rearing of sole larvae and juveniles

Incubation

Devauchelle et al. (1987) found that the best incubation performances (defined as the maximum hatching ratio and minimum % deformed newly hatched larvae) for *Solea solea* eggs was 13-15 ^{o}C, which is slightly higher than the optimal temperature for spawning found by the same authors (8-12 ^{o}C). Optimal salinity range for eggs and embryos is reported to be from 20 ‰ to

35 ‰ (Fonds, 1979; Devauchelle et al., 1987). The size of the eggs ranged from 1.0 to 1.6 mm, and has been reported to decrease during the spawning season (Houghton et al., 1985; Baynes et al., 1993). Fonds (1979) incubated sole eggs at five different temperatures between 10 and 22 °C and five different salinities between 20 ‰ and 50 ‰. High survival and normal development until hatching were observed at temperatures from 10 to 16 °C and salinities from 20 ‰ to 40 ‰. At 22 °C no viable larvae hatched and at 19 °C many embryos were abnormal. Survival was near 100 % at 10 °C. Incubation time (i.e., from fertilization to start of feeding) was highly dependent on the incubation temperatures and was 27.5, 19.7 and 15 days at 10, 13 and 16 °C, respectively. The optimal temperature for successful development of the eggs was lower than the optimal temperature for growth of the larvae, as maximum growth rates were found at 19 and 22 °C.

Saleh et al. (2016) reported that, hatching of *Solea aegyptiaca* eggs occured after 48±3 h from fertilization at temperature 17-19 °C, while occured after 60-72 h from fertilization at temperature 14-16 °C. Zaki et al. (1998) who reported that, the incubation period of eggs of *Solea aegyptiaca* at a temperature ranged from 16-18 °C is 48±6 h. Also, Herrera et al. (2008) reported that, incubation lasted for 36-48 h for wedge sole at 19 °C. Similarly, Salivatori *et al.* (1985) reported that hatching of *Solea* eggs occured after 45-46 h at temperature 16-18 °C. Also, Zaki and Hamza (1986) incubated *Solea solea* eggs at temperature ranged between 11-17 °C and hatched within 72-96 h. On the contrary, Assem et al. (2012) recorded that, *Solea vulgaris* eggs hatched after 38±2 h from fertilization at temperature of about 17±2 °C. Also, Yufera et al. (1999) recorded that, *S. senegalensis* eggs hatched after 38 h from fertilization at temperature of about 19.5 °C. In addition, Bedoui (1995) reported that hatching of *Solea senegalensis* eggs occured after 42 h at temperature 19 °C. Moreover, Ramos (1986c) showed that egg incubated at 20 ±1 °C and hatching occurred after 40-44 h. Also, Zaki and Hamza (1988) stated that incubation of *Solea solea* eggs varied from 3-4 days at 11-14 °C. Devauchelle et al. (1987) recorded that, *Solea solea* eggs hatched after 120 to 130 h from fertilization at temperature 13 °C. From these finding, it is concluded that incubation period varied for the same species with variation of water temperature. Also, there is difference in hatching period with other species of fish.

Salas-Leiton et al. (2012) assays carried out under laboratory-controlled conditions revealed that eggs of *Solea senegalensis* incubated at salinity 5, 10, 18, 27 and 33 ‰. High hatching rates (above 80 %) were obtained when incubating eggs for 48 hours at salinities of 10, 18, 27 and 33 ‰. Of special relevance was the finding that a salinity of 5 ‰ induced a 24 hours delay in the hatching embryos and that no egg hatching was obtained in the 0 ‰ group. Although an overall wide salinity range for successful egg development is a shared feature in estuarine-dependent flatfish, the optimum salinity for hatching might be considered to be species dependent.

Salas-Leiton et al. (2012) *Solea senegalensis* eggs incubated at salinity 5, 10, 18, 27 and 33 ‰. Yolk sac larvae showed no differences in survival from 4 DAH in relation to salinities in the range 10-33 ‰. This high tolerance is in agreement with results obtained for leopard grouper (0-40 ‰) (Gracia-Lopez et al., 2004) and in opposition to the narrower ranges described in species such as halibut (27-32 ‰) (Lein et al., 1997) and eel (24-36 ‰) (Okamoto et al., 2009). Although every group examined presented similar notochord lengths from 3 DAH, the myotomal height of yolk sac larvae grown at 10 ‰ salinity was significantly lower. Larger yolk sacs and an apparent delay in their consumption were also found in that group. One noticeable finding was the diverse jaw abnormalities (affecting 100 % of larvae) and the subsequent ingestion inability associated with a salinity of 10 ‰. As described in *Pagrus pagrus* (Ostrowski et al., 2011), notochord length was not affected by salinity. A similar inverse relationship between yolk sac volume and salinity in sole has been previously described in larvae of diverse species such as Atlantic herring (Holliday, Blaxter, 1960), Pacific herring (*Clupea pallasi*) (Alderdice, Velsen, 1971), pomfret (*Pampus punctatissimus*) (Shi et al., 2008) and halibut (Lein et al., 1997). Although a delay in yolk consumption affecting overall development could be inferred from this relationship, several authors have proposed higher water content, as a consequence of osmotic disequilibrium, to be responsible for the increased sac volume under reduced salinity conditions (May, 1974; Lein et al., 1997; Shi et al., 2008). The lower myotomal height measured in larvae reared at 10 ‰ salinity might also suggest differential energy mobilization associated with salinity; although such larvae would demand more energy to cope with an osmotically unfavourable external environment (Lein et al., 1997; Shi et al., 2008), the remaining salinity conditions (18, 27 and 33 ‰) would allow them to make more efficient use of the yolk resources to build body tissues. These general developmental anomalies are supported by the exclusive occurrence of jaw deformities in larvae reared at 10 ‰ salinity. Nutritional unbalance (Gisbert et al., 2008) and environmental conditions such as pollution (Sun et al., 2009), extreme temperature (Okamura et al., 2007) and physical stress (Morrison, MacDonald, 1995) have been reported as likely causes of jaw deformities. Knowledge about abnormalities in jaw development, and the resulting inability to feed (Morrison, MacDonald, 1995), induced by salinity is, however, more limited. Permanent gaping jaws syndrome has been characterized in Atlantic halibut larvae incubated at salinities ≤ 34 ‰ (Lein et al., 1997), whereas eel (Okamoto et al., 2009) and Pacific herring (Alderdice, Velsen, 1971) suffered delayed jaw development, abnormal lower jaws and gaping jaws in ≤33 and ≤25 ‰ salinity mediums, respectively. As the rearing water dilution was the only differential condition existing between our experimental groups, deficiencies in certain trace ions (e.g. zinc) (Somasundaram et al., 1984) might be considered to be the origin of the jaw malformations found in sole larvae raised at low salinity. Additional studies must be conducted to confirm this hypothesis.

Various types of experimental units have been used for incubation: rectangular 144 and 36 L tanks (Bromley, 1977), rectangular 120 L tanks (Fonds, 1979), rectangular 35 L tanks (Devauchelle et al., 1987), circular 20 L plastic tanks (Knutsen, 1992), rectangular 20 L glass tanks (Zaki et al., 1998), conical-based cylindrical tanks of 550 L capacity (Palazzi et al., 2006), cylinder conical 300 L tanks (Canavate et al., 2006), cylinder conical 80 and 500 L tanks (Blanco-Vives et al., 2010), square tanks of 280 L capacity (Bonaldo et al., 2011), cylinder conical 200 L tanks (Salas-Leiton et al., 2012) and rectangular glass aquaria of 25 L water capacity (Aljilany et al., 2016a). All experimental units provided satisfactory results.

Larval nutrition

Howell (1997) reviewed the culture of *Solea solea* and reported that rearing the larvae through metamorphosis presented few problems with survival rates being consistently in excess of 70 % in small scale laboratory systems. The larvae can be reared on a diet of freshly hatched *Artemia* nauplii without prior enrichment with algae or proprietary "booster" diets. The larvae have also been reared on a diet of rotifers, offered either as the exclusive food source (Howell, 1973) or in combination with *Artemia* nauplii (Fuchs, 1982).

The nutritional requirements for (n-3) HUFA in *Solea solea* is found to be less stringent than in many other marine species (Howell, Tzoumas, 1991) so that enhancing the lipid content of *Artemia* is not a prerequisite for high larval survival as long as *Artemia* strains rich in eicosepentaenoic acid, 20:5(n-3), are used. The effect of chemical stimuli on the feeding of *Solea solea* larvae has been investigated by Knutsen (1992). He found that the most potent substances in sole larvae were L-phenylalanine, L-lysin, L-asparagine, inosine 5-monophosphate and betaine. The results showed that sole larvae feeding are affected by chemosensory processes in the early larval stage and that chemoreception may be an integrated part of sole feeding strategy.

Dinis et al. (1996, 1999) described the first feeding in *Solea senegalensis* with larvae stocked at an initial density of 100 larvae/L. They reported that the larvae hatch with an average size of 2.4 ± 0.1 mm total length. The larvae were first fed on rotifers from 3 to 5 DAH along with *Artemia* nauplii from day 4 DAH onwards. During the larval period the environmental conditions were: normal photoperiod, a temperature of 18 °C and 35 ‰ salinity. Larvae accepted *Artemia* nauplii as first prey and reached a size of 8 mm by 15 DAH. Metamorphosis (i.e., start of eye migration) of started 11 days DAH and was completed 19 days DAH, with a total length of 7.3 ± 0.8 mm. From day 19 DAH the juveniles were fed on *Artemia* metanauplii (reared for 48 hours), and reached 16 ± 0.8 mm at day 40 DAH. Gavaia et al. (2002) tracked skeletal development during metamorphosis in *S. senegalensis*. Developing larvae were fed on *Artemia* nauplii from 3 to 10 DAH, 24 hours metanauplii from 11 DAH to metamorphosis, and 48 hours metanauplii until the end of the experiment (approximately 180 DAH). These changes were defined as (a) eye migration from left to right side and concomitant bending of the urostyle, and (b) torsion of internal organs that starts during the process of eye migration, this process was initiated in larvae around 4.1 mm in standard length (SL) and ended when larvae were approximately 8 mm SL.

Vazquez et al. (1994) investigated changes in biochemical composition and fatty acid content during the early development of *S. senegalensis* (10-12 hours after spawning, 36 hours after hatching and 5 DAH) as an indicator of the nutritional requirements during the early life stages. Saturated and monounsaturated fatty acids such as 16:0, 16:1*n*-7, 18:1*n*-9, and 18:1*n*-7 were utilized to a greater extent than polyunsaturated fatty acids as energy substrates. A requirement for longchain polyunsaturated fatty acids such as eicosapentaenoic acid, 20:5*n*-3, and docosahexaenoic acid, 22:6*n*-3, is likely since no evidence of bioconversion from their precursors was found. It seems that *Solea solea* and *Solea senegalensis* are less dependent on polyunsaturated fatty acids during early development compared to many other marine species (Howell, Tzoumas, 1991; Vazquez et al., 1994), thus enhancing the lipid content of *Artemia* is not a prerequisite for high larval survival.

Yufera et al. (1999) reported that, eggs and larvae of *Solea senegalensis* were reared from fertilization until the end of metamorphosis, which occurred at 17 DAH at 19.5 ºC. Changes in energy content and biomass quality were studied in terms of dry weight and nitrogen, carbon and energy content. *Solea senegalensis* spawned eggs of about 1 mm in diameter which hatched 38 hours after fertilization. The average dry weight of individual eggs was 46 µg, the chorion accounting for approximately 18 % of the total dry weight. Gross energy of recently fertilized sole eggs was about 1 Joule/egg. From fertilization to hatching the eggs lost 8 % of their total energy (chorion not included). After hatching, the larvae lost 14 % of their initial energy until the commencement of first feeding which occurred approximately 48 hours after hatching. The main components catabolized during embryogenesis were the carbon-rich compounds that were decreased by 26 %, while the nitrogen-rich compounds decreased by only 10 % and were practically unaltered from hatching to the onset of feeding. Larvae feeding showed consistent growth during the studied period (specific growth rate based on dry weight was 0.26/day). The relative proportion of nitrogen and carbon content revealed the accumulation of high energy compounds in the days before the metamorphosis.

Some time supplemented the initial rotifers diet with fertilized sea urchin eggs or a mixture of boiled egg yolk and finely-ground artificial feed (Nelson, Wilkins, 1994). Egg yolk, as a simple and available food, is a major source of minerals and vitamins being used to feed fish larvae newly absorbed their yolk sac (Maleknejad et al., 2014).

General chemical composition of poultry eggs

The weight and composition of a table egg depends on genetics, age, season, diet, etc. A typical White Leghorn egg usually weighs from 53 to 63 g with an average of 55 g (Ren et al., 2010). Board (1969) has shown that the main chemical compositions of hen egg are 11.8 % lipids, 12.8 % proteins, and small amounts of carbohydrates and minerals and in addition to water (74 %). Most of the proteins are found in the egg white and the egg yolk at 50 % and 44 %, respectively; the eggshell contains the rest of the proteins. The yolk a ccounts for slightly over one-third of the edible portion, but it produces three-fourths of the calories and provides all or most of the fat in a whole egg. Egg yolk consists of 48 % water, 32.6 % fat, 16 % protein, and some vitamins and minerals. The white consists of water (88 %), protein (10 %), and some minerals. The amount of lipid in the egg white is negligible (0.01 %) compared to the amount found in the yolk. The shell makes up 11 % of the weight of an egg, and approximately 98 % of the shell consists of calcium (Ren et al., 2010). Carbohydrates are a minor component of hen eggs. Their average content is about 0.5 grams per egg, 40 % of which is found in the yolk (Sugino et al., 1997). Carbohydrates are present as free and conjugated forms which are linked to proteins and lipids. Glucose accounts for about 98 % of the total free carbohydrate in the white. The carbohydrate content of egg yolk is about 1.0 %, and 0.7 % of it is consists of oligosaccharides bound to protein, composed of glucosamine and mannose; the remaining 0.3 % is free carbohydrate in the form of glucose (Ren et al., 2010). Nutrient composition of the edible portion of fresh raw hen's eggs and egg components are shown in (Tables 2, 3, 4, 5 and 6) (Ren et al., 2010).

Table 2. Chemical composition of fresh raw hen's eggs.[a]

Proximate nutrient	Whole egg	Egg white	Egg yolk
Water	37.66	29.33	8.1
Food energy (ca)	75	17	59
Protein (N×6.25) (g)	6.25	3.52	2.78
Total lipid (g)	5.01	--	5.12
Total carbohydrate (g)	0.61	0.34	0.3
Ash (g)	0.47	0.21	0.29

[a] Assayed nutrient values for large raw eggs based on 59 grams shell weight with 50 grams total liquid whole egg, 33.4 grams white, and 16.6 grams yolk.

Table 3. Lipids composition of fresh raw hen's eggs.[a]

Lipids	Whole egg	Egg white	Egg yolk
Fatty acids as triglycerides (g)	4.327	--	4.428
Saturated (total)	1.55	--	1.586
8:0 Caprylic	0.002	--	0.002
10:0 Capric	0.002	--	0.002
12:0 Lauric	0.002	--	0.002
14:0 Myristic	0.017	--	0.017
16:0 Palmitic	1.113	--	1.139
18:0 Stearic	0.392	--	0.401
20:0 Arachidic	0.02	--	0.02
Monounsaturated (total)	1.905	--	1.949
14:1 Myristoleic	0.005	--	0.005
16:1 Palmitoleic	0.149	--	0.152
18:1 Oleic	1.736	--	1.776
20:1 Eicosenoic	0.014	--	0.014
22:1 Erucic	0.002	--	0.002
Polyunsaturated (total)	0.682	--	0.698
18:2 Linoleic	0.574	--	0.587
18:3 Linolenic	0.017	--	0.017
20:4 Arachidonic	0.071	--	0.073
20:5 Eicosapentaeonic	0.002	--	0.002
22:6 Docosahexaenoic	0.018	--	0.019
Cholesterol (mg)	213	--	213
Lecithin (g)	1.15	--	1.11
Cephalin (g)	0.23	--	0.219

[a] Assayed nutrient values for large raw eggs based on 59 grams shell weight with 50 grams total liquid whole egg, 33.4 grams white, and 16.6 grams yolk.

Table 4. Vitamins composition of fresh raw hen's eggs.[a]

Vitamins	Whole egg	Egg white	Egg yolk
A (IU)	317	--	323
D (IU)	24.5	--	24.5
E (mg)	0.7	--	0.7
B12 (μg)	0.5	0.07	0.52
Biotin (μg)	9.98	2.34	7.58
Choline (mg)	215.06	0.42	216
Folic acid (folacin) (μg)	23	1.00	24
Inositol (mg)	5.39	1.38	3.95
Niacin (B3) (mg)	0.037	0.031	0.002
Pantothenic acid (mg)	0.627	0.04	0.632
Pyridoxine (B6) (mg)	0.07	0.001	0.065
Riboflavin (B2) (mg)	0.254	0.151	0.106
Thiamine (B1) (mg)	0.031	0.002	0.028

[a] Assayed nutrient values for large raw eggs based on 59 grams shell weight with 50 grams total liquid whole egg, 33.4 grams white, and 16.6 grams yolk.

Table 5. Minerals composition of fresh raw hen's eggs.[a]

Minerals (mg)	Whole egg	Egg white	Egg yolk
Calcium	25	2	23
Chlorine	87.1	60	27.1
Copper	0.007	0.002	0.004
Iodine	0.024	0.001	0.022
Iron	0.72	0.01	0.59
Magnesium	5	4	1
Manganese	0.012	0.001	0.012
Phosphorus	89	4	81
Potassium	60	48	16
Sodium	63	55	7
Sulfur	82	56	25
Zinc	0.55	--	0.52

[a] Assayed nutrient values for large raw eggs based on 59 grams shell weight with 50 grams total liquid whole egg, 33.4 grams white, and 16.6 grams yolk.

Table 6. Amino acid composition of fresh raw hen's eggs.[a]

Amino acids (g)	Whole egg	Egg white	Egg yolk
Alanine	0.348	0.203	0.143
Arginine	0.375	0.191	0.199
Aspartic acid	0.628	0.358	0.272
Cystine	0.145	0.091	0.05
Glutamic acid	0.816	0.467	0.353
Glycine	0.21	0.123	0.086
Histidine	0.148	0.079	0.072
Isoleucine	0.341	0.199	0.141
Leucine	0.534	0.296	0.244
Lysine	0.449	0.239	0.221
Methionine	0.195	0.121	0.069
Phenylalanine	0.332	0.205	0.119
Proline	0.249	0.137	0.116
Serine	0.465	0.242	0.238
Threonine	0.3	0.16	0.148
Tryptophan	0.076	0.043	0.033
Tyrosine	0.255	0.137	0.124
Valine	0.381	0.224	0.155

[a] Assayed nutrient values for large raw eggs based on 59 grams shell weight with 50 grams total liquid whole egg, 33.4 grams white, and 16.6 grams yolk.

Weaning

Total replacement of live food is still far to happen but the possibility of an early weaning of larvae using micro-diets could represent a valuable strategy to improving performance and reducing live food utilization. First-time feeding fish larvae usually have a rudimentary digestive system characterized by a non-functional stomach and low activity of digestive enzymes which make it difficult for them to process and assimilate a complex micro-diet (Kolkovski, 2001). The pre-weaning period has been greatly reduced due to conclusive results obtained in laboratory (Cahu, Zambonino-Infante, 2001). Furthermore, the use of a co-feeding regimen, which gradually weans larvae off live preys has been able to promote digestive maturation at early age (Engrola et al., 2007, 2009) and to improve the growth performance and survival rate of marine fish larvae (Rosenlund et al., 1997).

Bonaldo et al. (2011) studied the feasibility of weaning the larva's of common sole (*Solea solea*) at 13 DAH using commercial micro-diets while reducing live food utilization. Four DAH larvae were fed two types of experimental weaning feeding regimens (FRs): control FR-C (AgloNorse, Ewos) where larvae were weaned onto micro-diet at 27 DAH; whilst the other FRs provided live food until 13 DAH, employing AgloNorse, Ewos (FR-A), Gemma, Skretting (FR-G) and O.range Large, INVE (FR-O). The experiment lasted 29 days. No significant differences were observed between the groups in survival rates at the end of the experiment. FR-A, FR-G and FR-O showed significantly lower weight and length increase when compared to control, whereas metamorphosis development was not affected. In conclusion, a very early weaning at 13 DAH using commercial micro-diets in *Solea solea* larvae affects growth performance but not survival rate and metamorphosis development.

In the other studies on sole weaning, Engrola *et al.* (2007) reported that different weaning strategies on *Solea senegalensis* using the commercial micro-diets (AgloNorse). They obtained survival rates not exceeding 40 % at 26 and 33 DAH, but up to 90 % when larvae were weaned at 40 and 60 DAH. Interestingly, in the study conducted by Villalta et al. (2005), larvae of *Solea senegalensis* fed on the diet containing the highest concentration of docosaesaenoic acid (DHA), had the lowest growth in length compared to the other groups. They suggest that an increase in dietary docosaesaenoic acid and a reduction in monounsaturated fatty acids, specially 18:1n-9, may have led to reduced energy availability and subsequently reduced growth.

Palazzi et al. (2006) studied the three weaning trials for *Solea solea*, comparing two commercial feeds were carried out on larvae about 30 DAH. One of these feeds was sufficient in itself to complete the juvenile weaning, with survival rates reaching 85 %, which are comparable to those obtained in the control groups fed on live *Artemia*. 43 % survival rates were obtained with the second commercial feed. Both commercial feeds enabled superior growth of juvenile on average compared to the control groups.

Bromley (1977) studied eight different diets and four different methods of weaning for sole during the 25-40 day after hatching (about 13 mm at the start), and the best results were obtained from using a dry salmon feed as a weaning diet, achieving an average growth of 0.33 mm/day. Gatesoupe and Luquet (1982) fed sole larvae with live *Artemia* nauplii during the first few days of their life. The fish were then fed an artificial diet, which was semi-moist and crumbly in form. Good results were obtained without the addition of frozen nauplii (15 % and 28 % survival from hatching until day 70, respectively, for two lots abruptly weaned from day 10 or 11, with 50 and 90 mg as final weight, and supplying of 1980 or 2660 live nauplii per 70-day-old fish). The percentage of normally pigmented sole at the end of the trials was between 13 % and 70 %. The differences between lots of the same spawning were found to be significant. This may indicate that unchecked rearing conditions may lead to a very high level of pigmentation anomalies, a phenomenon that is frequently observed in the rearing of flatfish, e.g., in turbot (Heap, Thorpe, 1987) and halibut (Hemre et al., 2002). Fuchs (1982) obtained a mean weight of 400 mg in 60 day old sole, weaned by day 25, with 40 % survival from this date. This survival rate might be expected with weaning prior to metamorphosis, but one would expect the growth rate to be 5-10 times lower, and the amount of live nauplii to be more than 12 times lower. According to Gatesoupe and Luquet (1982) there is a choice to be made between two methods of weaning (i.e., *Artemia* for 25 days, Fuchs, 1978 or *Artemia* for 5-15 days supplemented with an artificial diet, Gatesoupe and Luquet, (1982). The first gives higher growth but also high costs (due do *Artemia* expenses), and the second alternative is less costly but produces lower growth. In a later study Gatesoupe (1983) weaned sole from live *Artemia* onto a compound diet between 10 and 15 DAH, and by 70 DAH the weight was between 189 and 414 mg, which, according to the author, is comparable to growth achieved in weaning studies with *Artemia* up to day 25. By choosing this strategy, a 90 % reduction in number of nauplii offered can be achieved, i.e., 3000 nauplii per surviving fish, compared to 25,000 nauplii per sole surviving in Fuchs (1978) study.

Cadena-Roa et al. (1982) studied the use of attractive substances in feed to increase the feed intake of sole during weaning. They used rehydratable extruded pellets because these are soft, palatable, and resistant to leaking and can be sized. Furthermore, an attractant can easily be added to these pellets. During a 45-days trial, extruded pellets containing only water, vitamins and oils were compared to pellets mixed with the following additives: ground Artemia, ground polychaetes, ground molluscs, mixture of glycine, L-alanine, L-glutamaic acid, L-arginine, betaine and inosine. As a result, the crumble absorbed the substances they were mixed with it. Supplementation with ground Artemia, molluscs or polychaetes did not result in better growth (specific growth rate, 1.5-3.0 % per day) and survival (10-17 %) than in the control (0.9 % and 3.0 %, respectively), whereas the mixture of chemical substances greatly enhanced those parameters (4 % per day and 66 %, respectively). Similar findings were reported in the study of Metailler et al. (1983) where inosine, glycine and betaine gave the best results as attractants. Further, Day et al. (1997) found that survival during the weaning period was positively correlated to the level of hydrolyzed fish protein concentrate, but they found no correlation with growth.

Appelbaum (1985) reared sole larvae from first feeding until metamorphosis on *Artemia* nauplii, inert diets, or inert diets following pre-feeding with *Artemia*. The duration of the pre-feeding ranged from 0 to 10 days. The growth and development of the receiving larvae were monitored for different diets. The inert diets were prepared as microcapsules or as ground and sieved particles. The results showed that sole larvae could be reared from first feeding until metamorphosis exclusively on inert diets. The longer the larvae were fed on *Artemia*, the greater were their rates of growth and development, but their mortality rate during weaning for a formulated diet was also higher. The best survival rate was obtained with an artificial diet when live *Artemia* nauplii were offered for the first ten days. Appelbaum (1985) concluded that a certain proportion of a population of sole can be reared from eggs to juvenile fishes fed only a formulated diet.

In conclusion, weaning has been started at different days post hatch, from 10 DAH (Gatesoupe, Luquet, 1982) and 13 DAH (Bonaldo et al., 2011) to 25-40 DAH (Bromley, 1977). Studies indicate that a mixture of inert and live food may increase the weaning attractants in the dry feed (Cadena-Roa et al., 1982; Metailler et al., 1983).

Substratum

Champalbert et al. (1992) investigated the influence of sediment on the settlement of juvenile sole (6-44 mm). Sediments of different grain size were used: mud (50-60 µm), fine sand (80-100 µm), coarse sand (200-250 µm) and gravel (1250-2500 µm). No differences were found in growth or survival. Sole sensitivity to sediment grain and/or colour varies with age. Larvae metamorphosing, or very recently metamorphosed juveniles, prefer settling on bright grounds, whatever the grain size. After metamorphosis, sole displays a tendency to settle on sandy substratum, preferentially on coarse sand or very fine mud. The tendency to choose bright sediment seems to decrease with increasing age. Moreover, soft ground is always preferred to hard bottom, even in animals never having experienced sand. Thus, sand appeared to be a suitable substratum. Although, sand is at present time not used to facilitate settlement in hatcheries, growth of metamorphosed larvae seems to be better in rearing units with sandy substratum.

Effect of temperature

Of all environmental factors that influence the growth rate in fish, temperature is the single most dominant (Imsland, Jonassen, 2001). Temperature is a rate-controlling factor for all chemical processes in poikilotherms. Irvin (1973) monitored the growth rate of hatchery reared juvenile *S. solea* of an initial mean total length of about 5 cm at five temperatures ranging from 11 to 27 °C for 12 weeks. The fish were fed *ad libitum* on an oligochaete worm. The fish showed an approximately linear increase in growth from 9 to 23 °C and a drop in growth after that. Fonds (1976) worked with wild-caught *S. solea* of a larger initial size (12-13 cm) and followed their growth for over a year at temperatures ranging from 10 to 25 °C. The fish were fed daily with fresh chopped mussel (*Mytilus edulis*) or live lugworm (*Arenicola marina*). In his study he found that the fish grew slower as they were larger than the fish in Irvin's (1973) study, but both experiments showed little increase in growth rates above 20 °C and indicated that the optimum temperature for growth (i.e., $T_{opt}G$) is between 20 and 25 °C. Howell (1997) extrapolated the data from these two studies and found that fish of about 5 cm may reach minimum market size of 24 cm (125 g) at temperatures close to optimum in less than 300 days.

Day et al. (1997) reared weaned juveniles for an 18-month period at an average temperature of 16.5 °C achieving a final average weight of 133g ± 40 SD (217.5 mm ± 19.1 SD). The study showed that intensive on-growing of sole may be performed in sand-free tanks, as a near market size of 22 cm may be obtained in 18 months with no mortalities. Growth of sole was negligible during the winter and started again in the spring, reaching a maximum incremental rate between May and June, at temperatures between 20 and 25°C (Palazzi et al., 2006).

Aljilany et al. (2016a) reported that, eight different temperatures (16, 17, 18, 19, 20, 21, 22 and 23°C) were tested for *Solea aegyptiaca* larvae. The larvae under temperature 20 °C showed the highest growth rate, while the larvae under temperature 16 °C showed the lower growth rate during the experiment. The growth rate for *Solea aegyptiaca* larvae has been increased gradually with increasing temperature until 20 °C then decreased. These results agreed with most studies on biological development and temperature in Senegal sole (*Solea senegalensis*) as many authors have used a constant temperature of 20 ºC (Martinez et al., 1999; Parra and Yufera, 1999; Ribeiro et al., 1999; Yufera et al., 1999; Canavate et al., 2006; Sanchez et al., 2010; Salas-Leiton et al., 2011, 2012). The Senegal sole is a flatfish adapted to temperate waters of around 20-21 ºC (Drake et al., 1984).

Blanco-Vives et al. (2010) reported that, Senegal sole larvae were exposed to constant temperature (20.7 °C), 22.1 °C day: 19.0 °C night and 19.2 °C day: 22.0 °C night. The sole larvae achieved the best growth performance, and fastest development under thermocycle conditions (22.1 °C day: 19.0 °C night). Larvae under (19 °C day/22 °C night) showed lower growth than those under the other treatments. In addition, Palazzi *et al.* (2006) recorded that, growth rate of *Solea solea* reached the maximum incremental rate between 20 and 25°C. Development of sole eggs succeed at temperature ranged from 7 to 19 °C, while, larval growth of sole was lower than that ranged from 10 to 23 °C (Fonds, 1979).

Aljilany et al. (2016a) showed a significant differences in completion of metamorphosis at 36, 36.5, 26.5, 22, 22, 15.5, 16 and 15.5 DAH for temperature 16, 17, 18, 19, 20, 21, 22 and 23°C, respectively of *Solea aegyptiaca*. Zaki et al. (1998) found that, the metamorphosis of *Solea aegyptiaca* was completed at 18, 27 and 29 DAH at 21.5, 20 and 15 ºC, respectively. Palazzi et al. (2006) and Lund et al. (2007) showed that, the metamorphosis of *Solea solea* was completed at 25 DAH at 18 ºC. Blanco-Vives et al. (2010) showed that, the metamorphosis of *Solea senegalensis* was completed at 17 DAH at (22.1 °C day: 19.0 °C night). And larvae reared under constant temperature (20.7 °C) completed the metamorphosis at 17 DAH, while larvae exposed to (19 °C day/22 °C night) completed at 19 DAH. Also, Dinis et al. (1996, 1999) showed that, the metamorphosis of *Solea senegalensis* was completed at 19 DAH at 18 ºC. As well as, Yufera *et al.* (1999) reared larvae of *Solea senegalensis* until the end of metamorphosis at 17 DAH at 19.5 ºC. While, Bonaldo et al. (2011) showed that, the metamorphosis of *Solea solea* was completed at 33 DAH at 18 ºC.

Aljilany et al. (2016a) reported that, survival rate values were relatively highest with larvae reared in 16 °C, while the larvae under temperature (22°C and 23°C) showed a lower survival rate. Survival rate decreased gradually with increasing temperature of *Solea aegyptiaca*. Similar results on *Solea aegyptiaca* were reported by Zaki et al. (1998) as survival rate through embryonic developmental stages were 83.3 % at 15 °C, 83 % at 16 °C, 82 % at 20 °C, survival rate was little decreased with increasing temperature. Also, Fonds (1979) incubated sole eggs at five different temperatures ranging from 10 to 22 ºC, high survival and normal development until hatching were observed at temperatures from 10 to 16 ºC. While, Blanco-Vives et al. (2010) reported that, *Solea senegalensis* larvae were exposed to constant temperature (20.7 °C), 22.1 °C day: 19.0 °C night and 19.2 °C day: 22.0 °C night. The sole larvae achieved the best survival under thermocycle conditions (22.1 °C day: 19.0 °C night).

Temperature has long been reported to influence the growth, survival and development of fish larvae both in the wild and laboratory (Methot, Kramer, 1979; Pepin, 1991; Green, Fisher, 2004; Johnston et al., 2004). In flatfish larvae the environmental factors may be crucial in determining recruitment to nursery grounds via their influence on growth and mortality during the metamorphosis-settlement period (Yamashita et al., 2001). Some studies have shown the existence of daily rhythms of temperature selection in fish in wild conditions. In such studies, fish showed daily migrations as they searched for a preferred temperature for physiological activity and growth (Gibson et al., 1998; Sims et al., 2006). Ottesen and Bolla (1998) reported that some physical parameters, including inappropriate temperature or salinity; it may be associated with jaw malformation due to mechanical damage in cultured larvae. The temperature in water undergoes changes according to the climatic fluctuations (Chapman, Kimstach, 1996). Surface water temperatures are affected by many different factors; season, altitude, latitude, time of day, cloud cover, air circulation and depth and flow of the body of water. Moreover, fish are more susceptible to disease at extreme temperatures (Holt et al., 1975). Provided that food availability is unrestricted, metabolic rate increases with increasing temperature. Growth is found to decrease at temperatures above the optimum because of a possible decrease in appetite and high energy cost of maintenance metabolism (Jobling, 1994). At low temperatures, growth is restricted because of low metabolic rates and low food intake. Hence, when culturing a species, best performance is obtained when temperatures are optimal.

Effect of photoperiod

Photostimulation affects fish growth through better food conversion efficiency, and not just through stimulated food intake (see review by Boeuf, Le Bail, 1999), but by increasing the production of somatotropin. Under changing photoperiods in commercial fish farming, fish are expected to gradually adjust from low metabolism under short day length, to higher feeding activity, growth, and food utilization under a summer photoperiod (Boehlert, 1981; Woiwode, Adelman, 1991). Both the direction and the rate of change in day length may influence these processes (Imsland, Jonassen, 2001). Seasonal variations in growth rate in wild populations of sole are known (Rogers, 1994), although the changes caused by photoperiod *per se* are difficult to isolate from other concurrent changes such as temperature. Moreover, studies on the effect of photoperiod on the growth of flatfish reared at constant temperatures are contradictory (Imsland et al., 2003).

The rapid physiological changes that *Solea senegalensis* larvae undergo throughout development, obtaining a fully metamorphosed stage at an age of 20 days (Fernandez-Diaz et al., 2001), make this species to be of particular interest for studying the effects of light during this period. It is hypothesised that the metamorphosis may also be affected by different feeding, growth and development responses under exposure to permanent photoperiod. To achieve such general goal, different specific objectives were targeted. Because *Solea senegalensis* is a usually nocturnal fish (Bayarri et al., 2004), the ability of the larvae to feed in the dark was initially monitored throughout the whole studied period. The potential effect on food ingestion of extending photoperiod from 14 light (L): 10 dark (D) to permanent illumination was also evaluated. The artificial photophase was adjusted to 14 hours since this is the local average of day-light duration following the main natural spawning period of *Solea senegalensis* (Anguis, Canavate, 2005).

Aljilany et al. (2016b) reported that, four different photoperiods (6L:18D, 12L:12D, 18L:6D and 24L:0D) were tested for *Solea aegyptiaca* larvae. Larvae exposed to photoperiods (12L:12D, 18L:6D and 24L:0D) led to higher length compared to 6L:18D. The highest final weight was observed in 24L:0D followed 18L:6D and 12L:12D then 6L:18D. The results indicated that the best growth performance of larvae at photoperiods of 18L:6D and 24L:0D. Canavate et al. (2006) reported that, growth of Senegal sole larvae was not significantly affected by photoperiods 14L:10D, 10L:14D and 24L:0D. Similar growth were reported for larvae of the close relative species *Solea solea* reared in 18L:6D or 24L:0D (Fuchs, 1978), and 15L:9D or 24L:0D (Ramos, 1986c). However, both authors reported slightly reduction in total length for larvae reared in 12L:12D and 11L:13D, respectively. Also, Leal *et* al. (2000) indicated similar lengths at 18 DAH for *Solea senegalensis* larvae reared in 12 hours or 24 hours light. Reports for flatfish also indicate some improvements in growth under longer photoperiods, although differences were never very high. Growth of 15 day-old *Paralichthys lethostigma* larvae was higher in 24L:0D and 18L:6D, in comparison to 12L:12D and 6L:18D (Moustakas et al., 2004). Growth of the summer flounder (*Paralichthys dentatus*) larvae was not affected when photoperiod was set to 24L:0D or 16L:8D (Huber et al., 1999). Reduction of photoperiod from 24L:0D to 12L:12D reduced growth in 20 day-old *Rhombosolea tapirina*, but 18L:6D produced similar results than permanent light (Hart et al., 1996). Growth was also lower for *Hippoglossus hippoglossus* larvae reared in 12L:12D, compared to 24L:0D (Solbakken, Pitmann, 2004). However, these authors found that eye migration to initiate earlier under the 12L:12D light regime. While, Tuckey and Smith (2001) found no photoperiod effect on growth after 54 days of rearing post-hatched *Paralichthys lethostigma*.

Aljilany et al. (2016b) showed that the metamorphosis period completed between 25.5 to 29 DAH under photoperiods (12L:12D, 18L:6D and 24L:0D), it was faster than 6L:18D (45 DAH). But 18L:6D results recorded the fastest metamorphosis (25.5 DAH) for *Solea aegyptiaca* larvae. Also, Blanco-Vives et al. (2010) showed that, metamorphosis was not complete until 27 DAH under photoperiod 12L:12D for *Solea senegalensis*. Contrarily, Canavate *et al.* (2006) for *Solea* *senegalensis* larvae exhibited similar metamorphosis under photoperiods (14L:10D, 10L:14D and 24L:0D) and completed from 19 to 20 DAH.

Aljilany et al. (2016b) reported that, *Solea aegyptiaca* larvae exhibited similar survival rate under photoperiods (12L:12D, 18L:6D and 24L:0D) and higher than 6L:18D. Also, Canavate et al. (2006) for *Solea senegalensis* larvae showed a similar survival rate under photoperiods of 14L:10D, 10L:14D and 24L:0D, Leal et al. (2000) for *Solea senegalensis*, and Fuchs (1978) for *Solea solea*, and other cultured flatfish larvae such as *R. tapirina* (Hart et al., 1996), *P. dentatus* (Huber et al., 1999) and *P. lethostigma* (Moustakas et al., 2004). Survival rate was higher in *H. hippoglossus* reared in 24L:0D against 12L:12D (Solbakken and Pitmann, 2004). On the other hand, Blanco-Vives *et al.* (2010) showed that, *Solea senegalensis* larvae reared under continuous darkness and continuous light died 15 and 17 DAH respectively at the beginning of metamorphosis. In addition, Tuckey and Smith (2001) found improved survival rate for *P. lethostigma* when photoperiod was decreased from 24L:0D to 10L:14D. Care must be taken when comparing photoperiod effects between species, as results are unavoidable influenced by different experimental conditions between facilities. Interactions due to the intensity of light (Downing and Litvak, 1999; Henne et al., 2001; Puvanendran and Brown, 2002; Moustakas et al., 2004), feed quality (Naess, Lie, 1998) or tank colour (Downing, Litvak, 1999).

Aljilany et al. (2016b) reported that, the better growth rate and faster metamorphosis of larvae were observed at photoperiods of 18L:6D and 24L:0D. Early pelagic stages of *Solea aegyptiaca* depended on light to capture rotifers. Rotifers and *Artemia* nauplii greatly differ in size, swimming activity and transparency, and these features may affect their detection capacity by larvae, especially in a low-light or no-light environment, as it has been described for *Solea senegalensis* (Canavate et al., 2006). In work (Mussi et al., 2005) different feeding reactions were described for a planktivorous fish larvae depending on transparency of the prey. There seems to be effect between food availability and light that improves larval growth rate (Boeuf, Le Bail, 1999). Permanent illumination improved growth rate of sole larvae. Increasing the duration of the visual feeding period is generally associated to a higher growth rate in cultured marine larval fish (Howell et al., 1998). Photoperiod may also stimulate hormones controlled by pineal organs which are responsible for circadian rhythms (Ekstrom, Meissi, 1997).

Aljilany et al. (2016b) reported that, the lowest survival rate, growth rate and slower development of larvae were observed at photoperiod of 6L:18D, as a possible consequence more reduced capacity of sole larvae to catch rotifers in the dark, larvae start to cease feeding at times coinciding with the onset of darkness, as it has been described for *S. senegalensis* (Canavate et al., 2006). The unfavorable photoperiods manifested in stress conditions that caused injuries and affected behavior, swimming activity, coloration, and growth (Mustapha et al., 2014). The unfavorable photoperiods could have also affected the immune systems of *O. niloticus* and *C. gariepinus* (Mustapha et al., 2014), Burgos et al. (2004) reported that artificial photoperiods affect the immune system of Rainbow trout, leading to mortality.

Effect of salinity

Marine teleosts have a blood concentration of ions lower than that of seawater (Zadunaisky, 1984), and regulate their internal osmotic pressure to a level equivalent to a salinity of 12-15 ‰ (Brett, 1979). Accordingly, marine fish species expend a certain amount of energy to meet the metabolic cost of ionic and osmotic regulation, and it has been hypothesized that if the external environment was manipulated so that the costs of ionic regulation was lowered, then the growth and food conversion efficiency of the fish would be improved. Furthermore, members of the Pleuronectidae family are sometimes found in freshwater (Evans, 1984), and some, like flounder, sole and turbot, may be considered euryhaline.

Cabral and Costa (1999) investigated the spatio-temporal pattern of estuarine use in the Tagus estuary of both *Solea solea* and *Solea senegalensis*. The authors found the highest densities of *Solea solea* in deep, warmer, low-salinity areas, while *Solea senegalensis* had a wider distribution and its abundance was more related to food availability. Champalbert et al. (1994) showed that *Solea solea* juvenile can tolerate low salinities and show an increased rheotaxis during periods of low salinity. As such this may indicate that salinity can influence the feeding behaviour or/and growth dynamics of sole. For turbot (Imsland et al., 2001, 2002) rearing juveniles at intermediate salinities improved growth by 10-15 % compared to full salinity. Whether similar results can be obtained in sole is unclear, but this is clearly an area for future research.

Aljilany et al. (2016a) reported that, five salinities (10, 15, 20, 25 and 30 ‰) were tested on *Solea aegyptiaca* larvae and found no significant difference in growth, but the best performance of larvae was at salinity 20 ‰. Salas-Leiton et al. (2012) who reported that, larval rearing until complete metamorphosis under standard culture conditions demonstrated that *Solea senegalensis* larvae could be successfully reared at 10 ‰ if transference to this low salinity is carried out once the mouth opening process has been completed (2-3 DAH). They added no significant differences were found between salinities of 10 and 33 ‰ when the feeding activity was analyzed in the first six days of rearing. Both salinities led to similar individual dry weights throughout the experimental period, with significantly higher weights recorded in larvae grown at 10 ‰ salinity only at 21 DAH. With the exception of 14 DAH, the higher weight achieved at 21 DAH in our larvae reared at 10 ‰ salinity is probably associated with decreased survival, and consequently with lower stocking densities during the last days of metamorphosis (Salas-Leiton et al., 2012). Also, similar results in the flatfish European flounder (*Platichthys flesus*) were obtained by O'Neill et al. (2011) who found that, no significant difference in ontogenetic development between exposures (salinity of 0, 10, 20 and 30 ‰). No significant differences in somatic growth rate, somatic condition or standard length were observed between treatments.

The relationship between salinity and growth rate throughout the larval stages in the flatfish, both Brazilian and greenback flounders showed optimum growth over salinities ranging from 20 to 30 ‰ (Sampaio et al., 2007) and from 15 to 35 ‰, respectively (Hart et al., 1996). The spotted halibut (*Verasper variegatus*) preferred moderately low salinities (8-16‰) during the larva-juvenile transformation period (Wada et al., 2004). Turbot inhabiting the North Sea had optimum rates at salinities above 20 ‰ (Karas, Klingsheim, 1997).

Aljilany et al. (2016a) showed that under experimental conditions of salinity, there were insignificant differences between treatments in the development of metamorphosis, a process completed 32.5-36.5 DAH under different salinities. Salas-Leiton et al. (2012) who showed that, salinity did not induce differences in the development of metamorphosis between 10 and 33 ‰ salinity, but a process completed 21 DAH under both salinities for *Solea senegalensis*.

Aljilany et al. (2016a) reported that, survival rate values were relatively highest with larvae reared in 20 ‰, while the larvae under 30 ‰ showed a lower survival rate, with significant differences between different salinities. Similarly, Moustakas et al. (2004) found that, larvae of southern flounder (*Paralichtys lethostigma*) showed reduced survival and markedly lower growth rates at full-strength seawater (35‰) compared to that achieved at a lower salinity (25 ‰) from hatching to 15 DAH. Also, Kerstan (1991) found that within estuaries, densities of juvenile *P. flesus* significantly increased with decreasing salinity. On the other hand, a higher final survival rate was obtained in *Solea senegalensis* larvae cultivated at 33 ‰ compared to 10 ‰ salinity (Salas-Leiton et al., 2012). Also, lower survival rates were obtained in greenback flounder larvae reared at 15 ‰ salinity in comparison to either 25 or 35 ‰ (Hart et al., 1996). Moreover, Salas-Leiton et al. (2012) found that, newly hatched and early-developing yolk sac larvae presented similar survival rates 3 DAH when exposed to salinities of 10, 18, 27 and 33 ‰, one noticeable finding was the diverse jaw abnormalities (affecting 100 % of larvae) and the subsequent ingestion inability associated with a salinity of 10 ‰.

Many studies in the literature have reported responses to salinity under laboratory conditions that are at odds with observations from wild populations. For example, three estuarine dependant species, the dusky kob (*Argyrosomus japonicus*), Brazilian flounder (*Paralichthys orbignyanus*) and spotted grunter (*Pomadasys commersonnii*) did not show enhanced growth rates under hyperosmotic conditions, and in all cases appeared to do better at higher salinities (Bernatzeder et al., 2010; Deacon and Hecht, 1999; Sampaio et al., 2001). Although laboratory experiments are not representative of the natural environment, studying the effects of an environmental variable in isolation can complement the interpretation of field studies. The growth and mortality responses of flounder vary between stable and fluctuating salinity conditions (Andersen et al., 2005); therefore, an experiment with varying salinity exposures would more closely simulate the natural tidal estuarine environment and may provide a better indication of the optimal salinity conditions for growth, condition and survival of flounder during metamorphosis.

Effect of stocking density

Although density is a key element in rearing of sole, few systematic investigations exist for this species. Howell (1998) described the effects of stocking density on the growth of turbot and

S. solea. In his study, conditions were created that minimized the effects of water quality to enable social effects to be quantified. Growth of turbot was unaffected until the combined area of the fish exceeded 200 % tank bottom coverage. In sole, growth was inversely related to stocking densities although percentage cover ranged from only 5 % to 130 %. It was postulated that the differing response to crowding in these two species could be related to their feeding behaviour. In contrast to turbot feeding behaviour, sole are adapted to eating small but frequent meals. This type of behaviour provides greater opportunities for interaction between individuals within communal groups than what is typically displayed in turbot, which is readily satiated by a relatively small number of feeding opportunities. This may indicate that sole is less well suited to intensive culture conditions than some other species. An experiment in which sole was grown from a mean length of 5 cm (1.5 g) to 10 cm (10-13 g), under conditions designed to quantify social rather than water quality effects on growth, demonstrated a significant negative effect of stocking density on growth (Howell, 1997).

Hierarchical effects and other social interactions were more apparent in sole as the oligochaete worm (*Lumbricillus rivalis*) they were fed with was concentrated in a small number of clumps in each tank leading to dominance hierarchies. It is possible that if fed formulated pellets, which are more evenly distributed in the tanks, this dominant behaviour would have become less prominent. Further studies on the effects of feeding strategy and stocking density are needed (Imsland et al., 2003).

Aljilany et al. (2016b) reported that, four different stocking densities (50, 80, 110, 140 larvae/liter) were tested for *Solea aegyptiaca* larvae. The growth rate of larvae was similarly in all stocking densities, without any statistical difference between treatments. Stocking density of 110 larvae/liter led to relatively higher survival rate compared to the other stocking densities. Salas-Leiton et al. (2008) who studied the effect of four stocking densities between 2 and 30 kg/m^2 of Senegalese sole that did not find any significant differences in biomass production or growth rates. Also, Salas-Leiton et al. (2010) studied the effect of stocking density (7 and 30 Kg/m^2) on the growth of Senegalese sole (*Solea senegalensis*) juveniles for 60 days, and there were no differences in specific growth rate between densities.

On the other hand, Schram et al. (2006) studied the effect of stocking density (0.5, 1.1, 5.1, 7.4, 10.2 and 12 kg/m^2) on the growth of Dover sole for 55 days, and they found that the SGR was decreasing with increasing stocking density. Survival rate decreased significantly with increasing stocking density for Dover sole.

Aljilany et al. (2016b) showed that growth of *Solea aegyptiaca* larvae was reasonably good at different stocking densities (from 50 to 140 larvae/liter). The findings may indicate that stocking density might have a limited effect on larvae growth during the intensive larval rearing period. The higher survival rate at high stocking density could be attributed to diminished social dominance (Kapinga et al., 2014).

Water quality aspects in intensive sole rearing

Production of sole in Northern Europe will take place in land-based facilities supplied with water pumped from depths of stable, optimal temperatures for the species or using heated seawater. An efficient exploitation of the water resources require detailed knowledge on the impact of water quality on fundamental production characteristics such as growth performance, food conversion efficiency and health status. Few studies have addressed the impact of a degraded water quality on the performance of sole. In intensive systems, i.e., shallow raceways in racks, where water may be reused between levels (Oiestad, 1999), fish densities may be higher than 500 Kg/m^3 (Oiestad, 1999), thus there will be a downstream gradual decrease in dissolved oxygen and pH, concurrent with a gradual accumulation of natural catabolites such as ammonia and carbon dioxide (Person-Le Ruyet et al., 1997a). These water parameters may separately or together affect production characteristics.

Ammonia is the main end product of nitrogen metabolism in teleosts (Foster, Goldstein, 1969), and it exists in both ionized ($NH^+{}_4$) and unionized (NH_3) forms. The toxicity of ammonia to fish and other aquatic organisms is primarily attributed to the unionized (UIA) form, and in intensive reuse systems, ammonia concentrations may increase to levels that can cause reduced growth or even death (Person-Le Ruyet et al., 1997a). In addition to being highly toxic alone, ammonia interacts with oxygen, to become more toxic at low levels of dissolved oxygen (DO) (Lloyd, 1961; Alabaster et al., 1979; Thurston et al., 1981; Wajsbrot et al., 1991). Concurrent with a gradual decrease in oxygen content downstream in a raceway, there will also be an inversely correlated increase in carbon dioxide (CO_2) content, which in turn will reduce pH. Alderson (1979) investigated the interactive effects of pH and chronic ammonia exposure on growth in juvenile *S. solea* and estimated the NOEC (No-Observable-Effect-Concentration) of UIA to be 0.066 mg/l. Above this level growth decreased in a linear manner with increasing concentrations of UIA. He also found that the different pH conditions used, ranging from 6.85 to 8.1, did not have any marked effect on this threshold limit. The UIA NOEC for sole is lower than that found for other flatfish species. Kim et al. (1997) estimated the NOEC for flounder, *Paralichthys olivaceus*, larvae to be 0.102 mg/l UIA and in turbot juveniles the NOEC has been found to be even higher, ranging from 0.09 to 0.18 mg/l UIA (Alderson, 1979; Rasmussen, Korsgaard, 1996; Person-Le Ruyet et al., 1997b). Parra and Yufera (1999) investigated tolerance to ammonia and nitrite in *S. senegalensis* larvae and found an UIA 24-h LC_{50} value of 1.32 p.p.m. No mortality occurred when *S. senegalensis* larvae were exposed to nitrite concentrations of up to 2000 p.p.m. for 24 h. *S. senegalensis* proved to be more resistant than the simultaneously studied gilthead seabream, *Sparus aurata*, and the increased tolerance to ammonia and nitrite in sole was associated with a greater amount of mucus in their skin. In the work of Parra and Yufera (2002), tolerance to water pH was investigated in *S. senegalensis* larvae. They found that low 24-h pHL_{50} values ranged between 4.88 and 5.76, whereas high 24-h pHL_{50} values ranged between 8.94 and 9.57.

Dissolved oxygen (DO) is, besides food and temperature, the most important factor controlling growth in fish, and a constant DO content below a critical level is considered to reduce food consumption, growth rate and food conversion efficiency (Jobling, 1994). The development of oxygen supplementation systems has alleviated some problems associated with low DO, but in a shallow raceway system, where fish densities may be extremely high, the reduction in DO content from inlet to outlet will be far greater than in a traditional tank system. In the natural environment, motile species can detect and avoid low levels of DO (Bejda et al., 1992), whereas in a culture facility, fish are inadvertently confined to the ambient DO levels determined by water-flow and fish biomass present. Significant effort has been put into determining threshold oxygen values for reduced growth rate and mortality in various cultured species (e.g., Bejda et al., 1992; Van den Thillart et al., 1994; Thetmeyer et al., 1999; Pichavant et al., 2000). Van den Thillart et al. (1994) investigated *S. solea* sensitivity to long-term hypoxia. They found that the resting metabolic rate and the scope for activity in the species showed significant changes at reduced oxygen levels. They suggested that the limiting O_2 level for *S. solea* was between 40 % and 60 % air saturation, while the incipient lethal level, indicated by the onset of anaerobic metabolism, was between 12 % and 20 %. Dalla Via et al. (1998) described the behavioural responses of sole to hypoxic conditions and concluded that escape behaviour and burst activity are induced as a last response when other energy-saving alternatives (anaerobic metabolism and metabolic depression) seem to become insufficient.

Protein requirements of sole

Focusing on the nutrition and feeding of Senegalese sole, which seem a particularly critical key issue, several significant advances on nutritional requirements have been reported (Aragao et al., 2003; Dias et al., 2004; Rema et al., 2008; Rubio et al., 2009). Other recent findings on juvenile *S. senegalensis* proved their high ability to use plant proteins and so offer good prospects for high levels of fish meal replacement (Silva et al., 2010; Dias et al., 2010). In these in vivo trials, fish were fed on diets with different protein levels or including plant protein sources, they mainly focus on obtaining zootechnical indexes (Rema et al., 2008; Silva et al., 2010), though Dias et al., (2010) also studied the apparent digestibility of several plant protein sources in this species. However, no information regarding the influence of these experimental diets on the digestive physiology of this species has been published so far (Rodiles et al., 2012).

The protein requirements for maximum growth of turbot, sole, plaice and Atlantic halibut ranges between 50 % and 65 % of diet (Berge, Storebakken, 1991; Guillaune et al., 1991; Aksnes et al., 1996). The dietary protein requirement for these flatfishes species during the juvenile stage is still scarce (Dias et al., 2004; Rubio et al., 2009). Some research has recently been achieved for *S. senegalensis* (Rema et al., 2008; Rubio et al., 2009) establishing optimal protein and lipid levels for growth. Less information is currently available for the common sole (*S. solea*) nutrition (Piccolo et al., 2008).

Yones and Abdel-Hakim (2011) studied the dietary protein requirement for optimal growth performance of juvenile sole *Solea aegyptiaca*. Four diets were formulated with different protein levels (D40, D45, D50 and D55 % crude protein). The results showed significant differences ($P < 0.05$) in growth performance and feed efficiency between diets. The highest performances were obtained with the D55 % CP, followed by D50 %, without significance difference between them. However, fish fed D40 % crude protein diet recorded less performance parameters.

3. Conclusion

Research on sole culture has increased over the last decade, and has extended our current knowledge of the species and promoted renewed interest in the aquaculture of sole species. As sole yet again is looked upon as promising aquaculture candidate, it is important to study the species in its natural environment, as optimal conditions in culture will try to mimic the optimal preferences of the species in nature. Broodstock productivity clearly represents the most significant constraint on commercial fish production. Increased knowledge of the factors regulating broodstock productivity is therefore of great importance to the further development of sole culture. Studies on sole have clearly indicated that, in contrast to other cultured marine fishes, fingerling production is not the bottleneck in proceeding towards commercial culture. This is mastered at laboratory and pilot scale, but experience with upscaling is still lacking. In sole culture, there are still obstacles in the development of feeding and ongrowing systems, which are mainly due to the peculiar feeding behaviour of this species. Currently, there is no commercial feed which suits the needs of sole and the farming technique used available. This review clearly indicates, that albeit there exists many unsolved problems and unanswered questions, sole remains a very attractive candidate for marine aquaculture and has a very big potential for future farming.

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