

## **The endocrine and metabolic interface of genotype-nutrition interaction in broilers and broiler breeders**

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### **Introduction**

Growth in broilers and in animals in general, is a very complex phenomenon determined by genotype as well as by environmental factors including nutrition. The neuroendocrine system acts as an interface to integrate the internal and external inputs. Hormones not only play an important role in the direct regulation of animal growth, both ponderal and proportional growth, but also contribute to the regulation of the interconversions of nutrients to metabolic substrates and their stored forms. Hormones help to regulate the levels of nutrients or metabolites by contributing to

- The control of absorption from the gut
- The regulation of blood metabolite levels
- The nature and rate of storage
- Their release from tissues
- Their assembly into structural elements of the body.

The nutrients that are absorbed from the digestive tract can be disposed of in the body in several ways. They may be used immediately as a source of energy, and the level of energy/heat production itself is to a large extent hormone dependent OR the nutrients that are not required for the immediate production of energy by the animal are stored, usually followed by their metabolic transformation.

### **Growth regulation**

Growth is here regarded as a kind of 'storage' in the broad sense. This storage is again dependent on genotype and environment, and a notable amount of the information by which these factors determine the nature of growth is again translated into hormonal changes that are the causal link between the factors on the one hand and the physiological processes leading to muscle, bone or fat growth on the other hand. It is well established that hormones regulating growth have different roles in different tissues. Amongst hormonal factors involved in growth regulation, the hypothalamo-pituitary-somatotrophic axis (GHRH/SS-GH-GHR) has received the greatest attention. As a consequence, since nutritional and/or genetic factors may alter the hormonal balance, hitherto they have a differential effect on different tissues.

An approach to investigate genotype-nutrition interaction was realized by Havenstein et al. (2003a,b). They showed that the 4.6 times increase in growth rate and 6-fold improvement in carcass yield of 2001 stocks of broilers fed a 2001 diet compared to 1957 stocks fed a 1957 diet is mainly (85 % or more) due to genetics and 10-15 % to nutritional changes. Another approach was to compare layers and broilers (Lohmann) both on pullet and broiler diet, almost isocaloric but with a 34 % higher crude protein, and 31 % and 10 % higher lysine and methionine respectively in the broiler diet. Growth as well as parameters of the somatotrophic axis was determined (Zhao et al., 2004). The body weight of layers on pullet diet (LL) was 417 g at 42 days and broilers on a broiler diet (BB) weighed 2576 g at 42 days. The body weight of layers increased with 35 % when fed the broiler diet, whereas that of broiler chickens decreased by 51 % when fed with the pullet diet. Plasma GH levels as well as the amplitude of the pulsatility was higher in layer chickens while the reverse was true for hepatic GHR mRNA. The differences between layers and broilers for all these parameters increased with age (from 5 to 42 days of age). The diet exchange completely reversed the

patterns of hypothalamic SS and pituitary GH mRNA expression and strain differences disappeared when the comparison was made on the same diet basis. The hepatic GHR mRNA decreased by 46.1 % in broilers fed the pullet diet, but increased by 45.6 % in the layer fed with broiler diet. While in the liver the strain differences for GHR-expression diminished with diet exchange, they did not completely disappear. However, muscle GHR mRNA expression was not affected by the diet exchange, indicating a genotype-specific determination of this characteristic. This can be linked with the observation of Havenstein et al. (1994) which showed that the improvement of growth in modern broilers to some extent is dependent on muscle responsiveness to GH, e.g. GHR-expression, is largely genetically determined.

The genotype-determined or diet-determined regulation of growth hormones or receptors such as GHR seems to be time- and tissue-specific. Genotype x diet interaction was also illustrated in somewhat older but very interesting experiments that showed that the diet-determined regulation of tissues (e.g. fat tissue) was genotype-specific. The amount of fat reduction by food restriction in fat versus lean birds depended on the nature of the selection parameter. While fatness could easily be reduced by restriction if fatness was the consequence of indirect selection (e.g. VLDL levels), this was not the case if fatness resulted from a direct selection (Whitehead, 1988; Whitehead and Griffin, 1984). In a more recent study that used genetically fat and lean lines of broilers (Leclercq et al., 1980), there was a combined effect of diet composition and genetic background of the broiler on the regulation of fat tissue. These chickens were reared on diets with isocaloric substitution between fat and protein levels. It was clearly shown that the broilers fed the low protein/high fat diet had significantly augmented proportional abdominal fat pad compared with the broilers on the low fat/high protein diet. Furthermore, the effect was more pronounced in the fat line resulting in a significant interaction between diet composition and genotype (Swennen and Buyse, personal communication).

### **Growth and Reproduction**

Genotype-diet interaction is not only present for growth parameters but even more accentuated in reproductive parameters and their endocrine control. Moreover, these genotype-diet interactions seem to have long lasting (or epigenetic) effects for the entire lifespan. The modern broiler breeder is indeed a compromise between two very different selection criteria : they need the genetics for rapid and efficient growth and have yet to exhibit a high rate of egg production (Renema and Robinson, 2004). The negative relationship between growth and reproduction fitness (Jaap and Muir, 1968) has been recognized for almost 40 years and similar relationships have also been shown in Japanese quail (Marks, 1985), turkey (Nestor et al., 1980) as well as in other species. Reproductive problems arising from decades of genetic selection for meat production traits have impaired reproductive ability of both broiler parents (Siegel and Dunnington, 1985). Limiting feed intake is currently the only practical tool to limit the negative effects of selection for growth in order to obtain an acceptable level of reproductive performance, hatching eggs and chick production. An obvious consequence of feed restriction is the potential for hunger and the impairment of welfare (including pecking, cannibalism, etc.) of the breeders (Hocking, 1993, 2004; Savory et al., 1999). With the decrease of the latter in commercial broiler breeder flocks, the use of feed restriction has become a husbandry problem that requires re-evaluation as to the duration and intensity. With the problems raised by the consequences of ad libitum feeding and those of restricted feeding, there is the need for alternative methods for producing heavy broiler breeders in order to maintain a balance between growth, good reproductive performance and the welfare of the breeders. Recent reports suggest that modifying the timing of restriction programs, the quantity/quality of the feed or the use of new genotypes that tolerate ad libitum feeding may achieve these objectives (Yu et al., 1992; Bruggeman et al., 1999, 2005 ;

Renema et al., 1999 ; Hocking et al., 2001, 2004 ; Heck et al., 2004 ; Jones et al., 2004). These authors provide evidence that, among others 1) limiting feed restriction to the rearing period alters the body weight gain and growth curve of the breeder hen and consequently improves egg production, welfare and survival rate; 2) Diet energy level dilution with high fibre (at low level) or a low energy food source has the potential to enhance welfare and maintain acceptable reproductive fitness 3) Broiler breeder hen crosses carrying the dwarf ('dw') gene, have the potential to tolerate ad libitum feeding and maintain a relatively lower body weight with high reproductive performance than standard heavy breeder hens.

In our laboratory, we have conducted several studies to determine the influence of nutrition level, feed quality manipulation, breeder genotype and their interactions on reproductive performance. The basis for differences in reproductive performance was also studied by comparing the body weights, ovarian follicular dynamics and reproductive hormone levels. In these studies, three heavy broiler breeder hen genotypes were compared : the Hybro G (Hybro bv, The Netherlands), a Standard broiler line (S genotype ; Hubbard-Europe, France) and an Experimental (E) genotype sex-linked dwarf cross (Hubbard-Europe). Day-old chicks of all genotypes were raised under standard conditions as recommended, on starter, grower and breeder diets. Two types of feeds were used : a normal commercial feed and an experimental energy-diluted feed that utilized wheat bran for energy dilution.

In a series of experiments, we determined

1. the effect of ad libitum feeding throughout (AAA), partial restriction (week 6-15 only ; ARA) and prolonged restriction (week 4-start of lay and during lay ; RRR) on body weight and egg production of breeders (Hybro G and S genotypes). In this experiment, commercial diets were used.
2. the effect of high energy diet (normal commercial feeds) and energy-diluted (experimental) diet on body weight and egg production of ad libitum fed and restricted broiler breeder hens. In this experiment, the AAA (of the S genotype) was compared with the RRR (S genotype) to determine the effect of energy intake levels.
3. the effect of dwarfing genotype on growth and reproductive performance. The S genotype was compared with the E genotype (dw) under ad libitum, partial (6-15 weeks) or total restriction using the energy-diluted diet.

Birds were weighed at regular intervals in order to determine body weight gains. Hens were caged at point of lay, to monitor egg production. At 32 and 42 weeks of age, a number of hens were slaughtered to count ovarian yellow follicle numbers. In protocol 3, blood sampling was conducted on 8 animals per group amongst the AAA and RRR to determine Luteinising Hormone (LH) and Progesterone (P<sub>4</sub>) levels in plasma during the ovulatory cycle.

Our data show a correspondence between food intake, body weight and reproduction. However, these findings also suggest that prolonged restriction may not be required to improve egg production in some genotypes. Furthermore, reproductive fitness thus seems to depend on the feeding management of heavy broiler breeders during the rapid growth period and it would seem that the intensity of body weight depression at this period is vital in determining its effect. The Hybro G showed greater depression and thus a better reproductive performance due to the different method of restriction (based on quantity : same quantity as restricted counterparts of the same age) compared to the S genotype (55% of food intake of ad libitum fed birds of the same age). The data from this study, along with those of Bruggeman et al. (1999), Hocking, (1993), Yu et al. (1999) in other genotypes affirm that manipulations of feeding during the growth period only, may be one of the potentials for improving reproductive fitness without compromising welfare.

The broiler breeder on the higher energy commercial diet tended to gain weight more rapidly than those on energy diluted feed. The final body weights of the AAA and RRR at 30 weeks

also tended to be higher in hens fed the higher energy diet. Surprisingly, even though there were very little differences in corresponding body weights under different regimes, egg production were significantly different. AAA hens on energy diluted feed had greater laying %; similarly the RRR also performed slightly better. Savory and Larivière (2000) also found no significant effect of energy dilution on body weight gains in different genotypes. The data on egg production from these experiments, seem to be a first report of such effect of feed energy dilution with fibre. However, other studies (Jones et al., 2004 ; Hocking et al., 2004) have shown that energy dilution with fibre enhances the welfare of ad libitum or restricted broiler breeders. Thus the improvement of egg production in this study along with the welfare effect may provide avenues for improving reproductive fitness.

The body weights and egg production records of the standard versus the dwarf cross genotype under the different feed regimes (AAA, ARA and RRR) were evaluated. Clearly there were significant differences in body weight gains or final body weights at 35 weeks between corresponding treatments. Partial restriction again reduced growth rate in the dwarf genotype as in the S genotype but the difference in the final weight between the AAA or ARA and the RRR was significantly greater in the S genotype. This indicates a significant effect of the dwarf gene in the E genotype and which may be a potential for limiting body weight gain of broiler breeders. Laying percentages were higher in the dwarf genotype in both AAA and ARA treatments. The data also show a better laying record for the dwarf genotype hens that were partially restricted compared to the RRR or AAA. Guillaume (1976), and Whitehead et al. (1987) have shown that the sex-linked 'dw' gene decreased body weight and that broiler breeder hen genotypes carrying the gene require minimum restriction to maximize egg output. Thus our results are consistent with these previous reports.

#### **Effect of feeding regimes on ovarian follicle dynamics and reproductive hormone levels**

Table 1 shows the number of yellow follicles on the ovary of genotypes according to the feeding treatments. Restricted feeding significantly reduced the number of follicles under both high and low energy feeds, especially during early lay (32 weeks). Energy dilution was especially beneficial in reducing follicle numbers in ad libitum fed hens during early lay. Yellow follicle numbers were lower in the AAA, ARA and RRR at both 32 and 42 weeks in dwarf compared with the S genotype. These data suggest that excessive number of yellow follicles can be prevented by restricted feeding, energy dilution or the use of the dwarf genotype. A major cause of reproductive dysfunction in heavy broiler breeders is the presence of excessive yellow follicle numbers which lead to irregular, erratic or multiple ovulations and atretic follicles (Hocking et al., 1987; Yu et al., 1999 ; Onagbesan et al., 1999). This pathology is often associated with the high body weights of hens fed ad libitum. The higher egg production of the ARA and RRR may be a consequence of the lower number of yellow follicles and more harmonious development of the hierarchy of follicles. Thus limiting body weight through the use of the dwarf genotype achieved similar aim as feed restriction.

#### **Genotype-Nutrition effect on reproductive hormones**

Blood plasma levels of LH and P<sub>4</sub> showed differences between genotypes and feeding treatments (table 2). The differences in LH levels were not as pronounced as those of P<sub>4</sub> levels. LH peak values were higher in the S-genotype compared to the dw-genotype and restriction increased both basal and LH peak values in dw but not in the S-genotype. On the other hand, P<sub>4</sub> values were higher in dw compared to the S-genotype for basal values while the fold increase to peak values was higher in the S-genotype. Restriction had a very pronounced effect on P<sub>4</sub> peak production in S-genotype but not in dw. Since P<sub>4</sub> production is an ovarian follicle function in response to LH, these data suggest an increased ovarian

sensitivity to gonadotrophins in restricted hens of the S genotype whereas there was no difference between the dwarf groups. This corresponded with the altered dynamics of ovarian yellow follicles in the S genotype and unaltered follicle dynamics in the dwarfs after feed restriction. These findings again point to genotype-dependent effects of feed restriction programs and their long-term effects. Our ovarian morphology data support the idea by Robinson that breeder hens maintaining more than 7-8 large yellow follicles at sexual maturity had a lower total egg production and higher incidence of unshetable egg production. Moreover, it further supports the idea of Bruggeman et al. (1999) that 7-15 weeks of age period may be the most critical time to restrict breeders because of the long term improvement in reproductive performance.

The differential responsiveness of different breeder genotypes to restriction has already been demonstrated by Onagbesan et al. (1999) in breeder female chickens selected for growth (GL-line) or feed conversion efficiency (FC-line). Feed restriction reduced body weights and increased egg production significantly and to a similar extent in both GL and FC lines. The number of normal and atretic yellow follicles was significantly higher under ad libitum feeding in the GL line than it was in the FC line. In culture, granulosa cells from the 3 largest follicles (F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub>) increased P<sub>4</sub> production in response to LH, FSH and IGF-1 but responses were different between the GL and FC lines fed either ad libitum or restricted diets. Responses were higher for FSH and IGF-1 stimulation in restricted GL-birds versus ad libitum birds while it was the reverse for the FC-line. Moreover, restriction of the GL-birds resulted in a simultaneously more pronounced difference in the 2 to 3 large follicles with high P<sub>4</sub> production in response to gonadotrophins or IGF-1, indicating a re-establishment of a kind of hierarchy in follicle differentiation by feed restriction.

## **Conclusion**

A solution to the dilemma of the appropriate feed management system to balance welfare and production concern in broiler parent stock is not readily available. As both ad libitum and restricted feeding programs have limitations with regard to welfare, any solution will likely be a compromise between these feeding methods. Therefore, a genotype capable of giving offsprings that grow rapidly while sustaining reproduction without the need for very heavy body weight and feed restriction and/or responding very well to moderate restriction during critical phases of development would be a solution for this dilemma. This points to the importance of genotype-nutrition interactions, together with epigenetics, in the long-term solution for the broiler breeder paradox.

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Table 1: Number of yellow follicles.

Genotype	Age (Wks)	Commercial Feed			Energy-diluted feed		
		AAA	ARA	RRR	AAA	ARA	RRR
Hybro G	32	10.8 ±0.9	10.0 ±0.9	5.7 ±0.23	ND	ND	ND
	42	7.2 ±0.7	6.4 ±0.3	6.4 ±0.4	ND	ND	ND
S-Genotype	32	11.0 ±0.09	ND	8.0 ±0.71	9.4 ±0.62	8.0 ±0.4	7.25 ±0.5
	42	5.25 ±0.89	ND	7.1 ±0.26	7.52 ±1.42	7.14 ±0.36	7.06 ±0.24
Dwarf	32	ND	ND	ND	7.2 ±0.24	6.1 ±0.12	6.2 ±0.44
	42	ND	ND	ND	6.54 ±0.88	6.20 ±0.36	6.45 ±0.42

ND represents Not determined.

Table 2: Plasma LH and Progesterone levels (P4)(ng/ml) during the ovulatory cycle of S- and Dwarf- Genotypes.

	LH				P4			
	S-AAA	S-RRR	Dwarf-AAA	Dwarf-RRR	S-AAA	S-RRR	Dwarf-AAA	Dwarf-RRR
Av. Basal before peak	2.84	2.34	2.15	2.75	0.115	0.103	0.276	0.240
Av. Peak	5.65	5.71	4.03	5.43	0.509	0.836	0.736	0.621
Fold increase between basal before peak and peak level	1.989	2.440	1.874	1.975	4.4	8.12	2.67	2.58