Unidentified Factors in Jojoba Meal Prevent Oviduct Development in Broiler Breeder Females

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Supplementation of feed with jojoba meal, as a means for autonomous feed restriction, was successful in depressing feed intake and controlling body weight of broiler breeder pullets to the extent recommended by the breeder company. However, these broiler breeders never produced eggs. At the level of ovary, normal follicle development and maturation did occur. A considerable number of ovulations occurred which were not followed by oviposition. After ovulation, the ova could not be captured by the oviduct, because of the small size of the oviduct, resulting in "internal laying". The virtual absence of oviduct development cannot be explained presently but it must be due to some yet unidentified factor(s) in jojoba meal which prevent(s) the normal development of the oviduct. These factors may be acting by abnormally increasing plasma progesterone or triiodothyronin levels and/or directly by themselves interfering with oviduct development. The nature of these factors requires further investigations.

Keywords: *Jojoba; broiler breeder female; oviduct; feed restriction*

INTRODUCTION

The jojoba plant (*Simmondsia chinensis*) is a native oilseed shrub of the Sonoran desert including parts of Arizona, California, and Mexico. The principal product extracted from the seeds is a liquid wax with characteristics similar to sperm whale oil (Verbiscar and Banigan, 1978). Jojoba oil is used as an additive in mineral oils and in cosmetics (Bagby, 1988). Jojoba meal, a byproduct of the oil extraction, contains approximately 30% proteins and represents a potential ingredient for animal feed, due to this high protein content. However, supplementing feed with defatted jojoba meal inhibits feed intake in rats (Booth *et al*., 1974; Cokelaere *et al*., 1993a), chickens (Ngou Ngoupayou *et al*., 1982; Arnouts *et al*., 1993; Vermaut *et al*., 1996, 1997a), sheep (Manos *et al*., 1986), mice (Sherbrooke *et al*., 1976), and rabbits (Ngou Ngoupayou *et al*., 1985).

To investigate the possible use of defatted jojoba meal—with its feed restricting characteristics—as animal feed, it has been tested in growing broiler breeder pullets. The feed intake of broiler breeder pullets must be restricted from a young age onwards in order to control body weight gain and hence to prevent leg disorders, high mortality rates, and excessive fattening. Concomitant with increases in the growth rate of broiler chickens, the ability of the meat-type parent stock to reproduce has been severely reduced. Feed restriction of the broiler breeders during rearing delays sexual maturity and improves egg production and fertility (Van Wambeke, 1981; Yu *et al*., 1992). There is an absolute necessity to regulate feed intake, in particular energy, during the rearing period. Quantitative regulation offers the greatest degree of control. With mechanical weight-scales, attached to automatic feeders, it is relatively easy to release accurate feed allocation under commercial conditions. Skip-a-day programs as well as everyday feed restriction can be applied. From the viewpoint of animal welfare the currently used practice of skip-a-day feed restriction methods is questioned and several country legislations already forbid fasting periods longer than 24 h. Nowadays, there is a growing concern about the welfare of broiler breeders, submitted to the severe physical feed restriction in typical commercial rearing programs (Hocking *et al*., 1996). Qualitative, self-restricting feeding methods would save labor or investment costs and minimize stress. Until now, no satisfactory autonomous feed restriction methods for practical use have been identified. However, Vermaut *et al*. (1997b, submitted) showed that supplementing the diet with jojoba meal was successful to establish an autonomous self-restriction by the pullets when fed *ad libitum* during the rearing period. The purpose of the present study was to determine the effects of jojoba meal supplementation on reproductive parameters of these broiler breeders during the laying period. Results were compared with those of quantitative restricted broiler breeders.

EXPERIMENTAL PROCEDURES

Jojoba Meal Preparation. Jojoba nuts were pressed in Israel at a temperature of 50 °C (Jojoba Israel, Kibbutz

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Hatzerim, Negev, Israel). The resulting jojoba press-cake, still containing 13.6 ± 0.5% fat, was deoiled by Soxhlet extraction for 8 h with *n*-hexane. After extraction, the fat content, measured by Soxhlet (1 h extracted with petroleum ether) was 1 ± 0.3%.

Animals, Housing, and Management. Seven hundred eighty 1-day-old female broiler breeder pullets (Ross 208) were raised in floor pens. During the first 3 weeks of age, all chicks had free access to a starter diet (11.7 MJ/kg metabolizable energy (ME); 18.2% crude protein (CP)). From 3 to 20 weeks, the pullets were restricted by two different methods as described below. From 20 to 64 weeks, all groups were fed a breeders' diet (11.5 MJ/kg metabolizable energy (ME); 17% CP; 3% Ca; 0.4% P available) and were quantitatively restricted in feed intake following the recommendations of the breeder company. Access to water was unrestricted. Temperature and light schedule were set according to the recommendation of the breeder company. The photoperiod was 23-h light and 1-h dark (23L:1D) during the first week of age and decreased to 8L:16D at week 6. The pullets were photostimulated from 19 weeks of age on by increasing weekly the photoperiod by 2 h up to 14L:10D at 21 weeks of age.

Experimental Design. At the age of 3 weeks, the pullets were divided into 12 groups of 65 chicks each. At this age, four groups were restricted quantitatively (R) in order to obtain a body weight curve as is recommended by the breeder company for broiler breeder females. They were fed daily on a restricted quantity of a chick grower meal (11.1 MJ ME/kg and 15% CP). These R groups were compared with four groups of pullets, provided the grower diet *ad libitum*, supplemented with jojoba meal (JO). Initially, supplementation was at 4% level (on the basis of Arnouts *et al.*, 1993) from 3 to 6 weeks, but the amount of jojoba meal had to be reduced at 8 weeks to 3.25%, at 10 weeks to 3%, and at 12 weeks to 2.5% to obtain the comparable body weight with the R group. The last four groups received an *ad libitum* control grower diet—without any supplementation—and served as the control group (C) for feed intake and growth.

Body weights and feed intake were recorded every week throughout the experimental period. Between 8 and 25 weeks, blood samples were collected every 2 or 3 weeks by wing vein puncture into heparinized tubes from six pullets randomly selected from each group of 24 chickens per treatment (after the daily meal of the R group). Plasma was separated by centrifugation and stored at −20 °C until analysis for plasma oestradiol (E_2) and luteinizing hormone (LH) at week 25 and plasma triiodothyronin (T_3) and progesterone (P_4) only at week 21 (because there were not enough plasma samples for detections at 25 weeks).

At the age of 27 and 35 weeks, six birds per treatment were sacrificed by cervical dislocation. Ovarian weight and oviduct weight and length were determined. Pituitary was taken out and stored at −80 °C until analysis for LH after homogenization.

Assessment of Ovary Development. Ovary development was assessed by hierarchical classification as described by Decuypere *et al.* (1993). In short, all clearly visible follicles were dissected and immediately weighed in order to classify them in weight classes. This allowed comparison for follicle size in JO and R groups. Subdivision into classes was achieved as shown in Figure 5. The presence of post-ovulatory follicles (F0) in both groups was also recorded.

***In Vitro* Progesterone Secretion.** At the age of 27 weeks, the ovarian follicles were removed and *in vitro* P_4 production from granulosa cells was determined. Granulosa cells were isolated as described by Gilbert *et al.* (1977) and cultured in M199 (500 μL) as previously established in our laboratory (Vanmontfort *et al.*, 1995). Cells were cultured for 24 h. Basal and LH-stimulated productions were determined to assess granulosa cell sensitivity to ovine pituitary LH. Media were collected and stored at −20 °C until assayed.

Comb and Wattle Development. A subjective observation of the size and shape of combs and wattles was made and photographs were taken from birds from the different groups.

Radioimmunoassays. *Triiodothyronin* (T_3). T_3 measurements were performed by radioimmunoassay using a commercially available T_3 antiserum (Mallinckrodt Diagnostica, Dietzenbach, Germany) in combination with a specific tracer (Amersham International, Slough, England). The intraassay coefficient of variation was 2.9% (Huybrechts *et al.*, 1989).

Steroid Hormones. Plasma progesterone and oestradiol concentrations were measured by RIA using the method developed by Cailleau (1979) and adapted for hens as described by Verheyen *et al.* (1987). Both steroids were extracted from plasma with cyclohexane/ethyl acetate (1:1, v/v). Intra- and interassay variabilities were 6.2 and 10.5% for P_4 and 5.3 and 9.9% for E_2, respectively.

The P_4 produced in the *in vitro* granulosa cell model was determined by using a commercially available RIA kit (ICN Biomedicals Inc., Costa Mesa) without extractions. The intraassay coefficient of variation was 5%.

Luteinizing Hormone (LH). An homologous RIA of cLH (Krishnan *et al.*, 1994) was used with reagents, supplied by Dr. J. A. Proudman and D. J. Bolt (USDA, Beltsville). Chicken LH (USDA-cLH-1−1) was radioiodinated by using a stoichiometric chloramine-T procedure (Proudman and Opel, 1981). The iodination mixture was purified by gel permeation on a PD-10 column (Pharmacia, Uppsala, Sweden). The RIA was performed under equilibrium conditions by using USDA-cLH-K-3 as a standard. Separation of bound and free radioactivity was attained by the addition of 100 μL of a second antibody-coated cellulose suspension (Innogenetics, Antwerp Belgium) per tube, the addition of 1 mL of distilled water after a 30-min incubation, centrifugation, and removal of the supernatant by aspiration. The lowest detectable level of LH was 0.1 ng/mL. The intra-assay coefficient of variation was 3.3%.

Statistical Analysis. Values are expressed as mean ± SEM. Statistical differences were tested between treatments by one-way ANOVA, followed by the least squares means test (General Linear Model procedure, SAS, 1986).

RESULTS

Growth and Feed Uptake Data during Rearing. Briefly, weekly cumulative feed intake was significantly higher in the C group than in both JO-supplemented and quantitatively restricted (R) groups. The C groups had significantly higher body weights at all ages than both JO and R groups, whereas the growth curves showed a similar trend between the R and JO fed groups.

Plasma Hormone Concentrations during Rearing. *Plasma LH.* Plasma LH data are summarized in Figure 1. From the age of 8 weeks, plasma levels increased with age in both R and JO fed pullets. Initially, between 8 and 12 weeks, levels in the JO pullets were significantly lower. LH levels in JO pullets increased rapidly from 12 weeks onward to level with those of R pullets. Plasma LH levels in the C group have not been measured.

Plasma P_4. Figure 2 shows the levels of plasma P_4 in the three groups from 8 to 21 weeks. Plasma progesterone levels were similar for the C, JO, and R pullets at 8 weeks. However, at 12 weeks of age, plasma P_4 levels in the JO group were significantly higher than in the C and R groups, between which no differences in P_4 have been observed. P_4 began to rise rapidly in the JO group between 14 and 16 weeks of age, while those of the C and R pullets remain significantly lower and similar and did not start to rise until between 16 and 18 weeks, when the level of P_4 in the JO group was already maximal. However, by 21 weeks, P_4 concentrations in the C pullets has risen to a similar level with that of the JO group. At that age, plasma P_4 concentra-

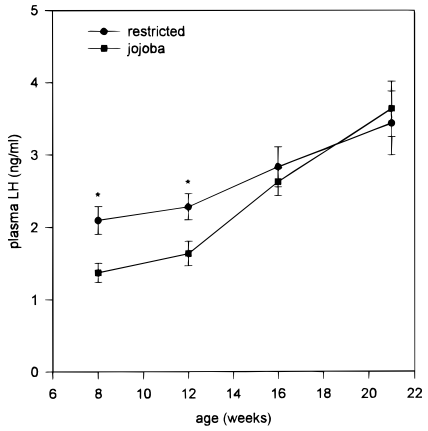

Figure 1. Effect of jojoba meal in comparison with a quantitative feed restriction (restricted) on plasma LH levels of female broiler breeder pullets at 8, 12, 16, and 21 weeks of age. Values are means from 24 chickens per dietary treatment and are expressed as means ± SEM. An asterisk indicates a significant difference between restricted and jojoba treatments at any age.

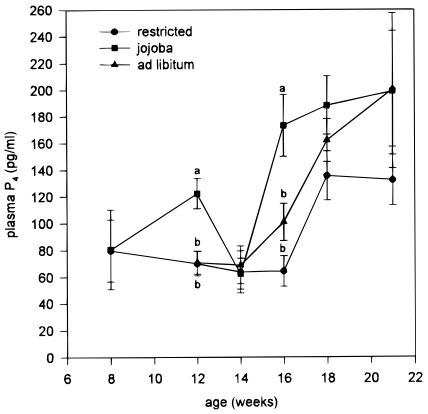

Figure 2. Effect of jojoba meal in comparison with a quantitative feed restriction (restricted) on plasma P_4 levels of female broiler breeder pullets at 8, 12, 14, 16, 18, and 21 weeks of age. Values are means from 24 chickens per dietary treatment and are expressed as means ± SEM. a and b denote significant overall treatment effect at any age ($P < 0.05$).

tion in the R group was lower than in both other groups, but this difference was not statistically significant.

Plasma E_2. Plasma E_2 levels were low and similar in all three groups up to 12 weeks of age. At the age of 14 weeks, plasma E_2 levels were significantly higher in the C group. From week 14 onward, E_2 levels were rising to similar amounts at 16 weeks and were maximal in all groups at 18 weeks (Figure 3). However, at 18−21 weeks, the levels of E_2 in the C group were significantly higher than in both the JO and R groups, in which E_2 levels were similar.

Plasma T_3. Plasma T_3 in all three groups studied decreased at 8−21 weeks. T_3 levels in the JO group were initially higher than in the C and R groups between week 12 and 14. This difference was only significant between the JO and the R group and this only at the age of 8 and 14 weeks. From 14 weeks onward, T_3 levels decreased in all groups until week 21. In the JO group, T_3 continued to decrease to is lowest level until week 23. At that age, T_3 level was increased again in the C and R group, resulting in a statistically significant difference between the three groups (Figure 4).

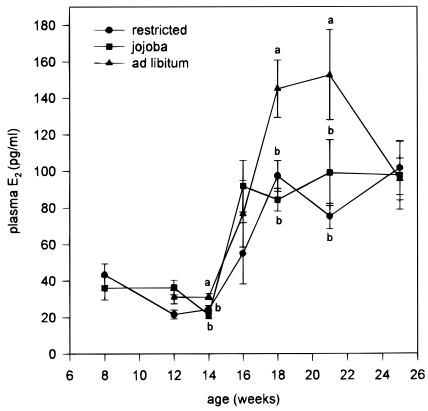

Figure 3. Effect of jojoba meal in comparison with a quantitative feed restriction (restricted) on plasma E_2 levels of female broiler breeder pullets at 8, 12, 14, 16, 18, 21, and 25 weeks of age. Values are means from 24 chickens per dietary treatment and are expressed as means ± SEM. a and b denote significant overall treatment effect at any age ($P < 0.05$).

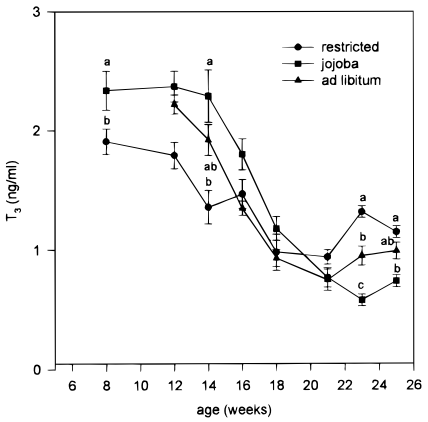

Figure 4. Effect of jojoba meal in comparison with a quantitative feed restriction (restricted) on plasma T_3 levels of female broiler breeder pullets at 8, 12, 14, 16, 21, 25, and 27 weeks of age. Values are means from 24 chickens per dietary treatment and are expressed as means ± SEM. a and b denote significant overall treatment effect at any age ($P < 0.05$).

Table 1. Ovarian Weights (g per kg of BW) at Different Ages of Quantitatively Restricted Pullets (Restricted) and Pullets That Received a Jojoba-Supplemented Diet at 3−20 Weeks of Age[a]

	ovarian weight		
age	restricted	jojoba	
27 wk	66.41 ± 2.70	58.34 ± 6.37	NS
35 wk	71.90 ± 6.33	59.80 ± 5.98	NS

[a] NS denotes no significant overall treatment effect ($P < 0.05$).

Egg Laying. The first eggs were laid between 20 and 23 weeks of age in the C group and between 24 and 27 weeks in the R group. In the JO group, birds did not lay eggs. C and R birds continued to lay throughout the duration of the study, whereas JO birds never started to lay.

Ovary Weight, Follicular Development and Distributions. At 27 and 35 weeks, ovary weight and follicular development and distributions were determined in the R and JO groups. This is summarized in Table 1 and Figure 5. Figure 6 depicts the ovary with the array of follicular developments and the states of the oviduct development *in situ*. Data on ovary weights show that the ovary had reached maximal growth by 27 weeks,

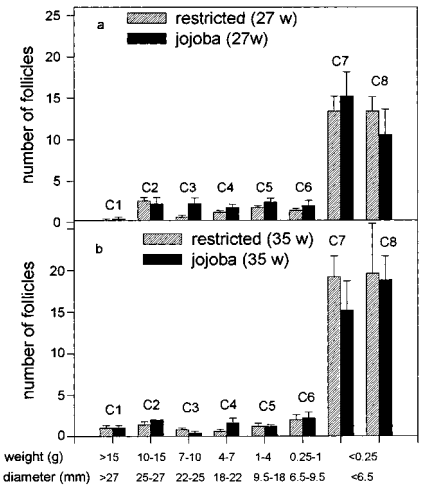

Figure 5. Mean follicle distribution per bird (six birds per treatment) and per class for jojoba and quantitatively restricted broiler breeder females. Follicle classes are expressed per weight (g) or per size (diameter in mm) and are expressed as means ± SEM.

as there was no further increase in weight at 35 weeks. There was no significant difference between JO fed and R birds at both 27 and 35 weeks.

Figure 5 shows data on the array of follicles (hierarchy) present in the ovaries of JO and R birds at 27 and 35 weeks of age. Data show that the ovaries of both groups contain similar classes of follicles at both time points. Although slight variations were recorded for some classes, especially the small white follicles, these differences were not significant. A count of postovulatory follicles on the ovaries of JO birds showed that ovulation did occur.

Figure 6 confirms normal development and full compliments of follicular hierarchy in the ovaries of JO-fed and R birds.

Weight and Length of Oviducts. Table 2 shows the weight and length of the oviducts of birds fed on JO-supplemented feed and those on restricted feeding, at 27, 35 and 60 weeks. Oviducts in the JO group barely developed. Weights were 17−20 times smaller than those of R birds at all ages. They were significantly shorter and slimmer, without maybe some differentiaton of magnum and isthmus. Plate 1 a and b show the oviducts of JO and R birds *in situ*.

In Vitro P_4 Production by Ovarian Granulosa Cells. Figure 7 shows that the granulosa cells from ovary of JO and R chickens produced progesterone and responded to LH stimulation. However, cells from JO birds were more responsive to LH than those from the R birds.

Pituitary LH Concentrations. There were no differences in LH concentration in the pituitaries between the JO (1.142 ± 0.153 μg/mg of pituitary) and R treatment (0.919 ± 0.080 μg/mg of pituitary) at the age of 35 weeks.

Comb and Wattle Development. Finally, Figure 8 shows that the comb and the wattle of the JO birds were not developed normally in comparison with a normal development of these of the R birds.

DISCUSSION

Inclusion of jojoba meal in the diet of female broiler breeder chickens restricts feed intake even when fed *ad libitum* and controls body weight gains to similar extents as conventional restricted quantitative feeding often recommended by breeder companies (Vermaut *et al.*, 1997b). The dose of jojoba supplementation has been reduced gradually in order to obtain the body weight curve of the R pullets by regulating feed intake. In this study, data on the subsequent reproductive performance of birds reared on diets supplemented with jojoba meal show that these birds did not lay eggs throughout the duration of the study, although they have followed the desired growth curve necessary for good performances. In our bid to elucidate the causal reasons for this defect, some of the parameters that are implicated in normal reproductive performances were studied. These include plasma LH, progesterone (P_4), oestradiol (E_2), and triiodothyronin (T_3) levels, ovary growth, follicular hierarchy and P_4 production in response to LH, and the development of the oviduct. Comparative analyses of the results with those of normally laying restricted (R) chickens and with the results of Hocking *et al.* (1989) show that ovary development was normal and contains a full complement of the range of follicles that would normally be present in the ovary. The presence of postovulatory follicles indicated that follicles ovulated internally. These data suggest that the ovaries of the jojoba-fed birds were normal in development and function and could not have been the cause of the defect in egg production.

Plasma levels of oestradiol were similar at all ages to those of the restricted birds that were laying normally. Both showed similar prepubertal increase in E_2 between 14 and 16 weeks. However, plasma P_4 levels were abnormally higher in chickens fed jojoba than in both *ad libitum* and restricted pullets. That apart, normal prepubertal increase that is often noticed in female hens rose 2 weeks earlier than in both C and R pullets although there were similar concentrations of LH in the plasma at this period. This higher P_4 could have resulted from what we perceived as the extrasensitivity of the ovary to the circulating gonadotrophins, if that was the source of the P_4. Indeed, our data on *in vitro* P_4 production from granulosa cells isolated from follicles showed that those cells obtained from pullets raised on jojoba meal synthesised more P_4 in response to LH than cells from the R pullets. It is suggested that the ovary of the birds on jojoba already had small yellow follicles at the period of development before sexual maturity. Alternatively, synthesis from the adrenals could be the source of this higher P_4. At present, we cannot explain why granulosa cells from the ovary of jojoba-fed birds produce more P_4 in response to LH. These findings implicate that jojoba is the component of the feed which is probably responsible for these alterations in P_4 production and that it might contain factors capable of altering the steroidogenic potentials of the follicular cells in the ovary of the chicken.

T_3 concentrations in the plasma were also significantly higher in the jojoba-fed pullets than in both C and R pullets during the rearing period. It declined to similar levels just before maturation but later by 2 weeks. We have reported previously that increased T_3 levels accompany jojoba supplementation in broiler rearing feeds (Vermaut *et al.*, 1997b).

The results show that the major primary cause of oviposition failure was the nondevelopment of the oviducts in the birds that were raised on jojoba meal. Whereas oviduct weights continued to increase in the R birds until 60 weeks, there was virtually no growth

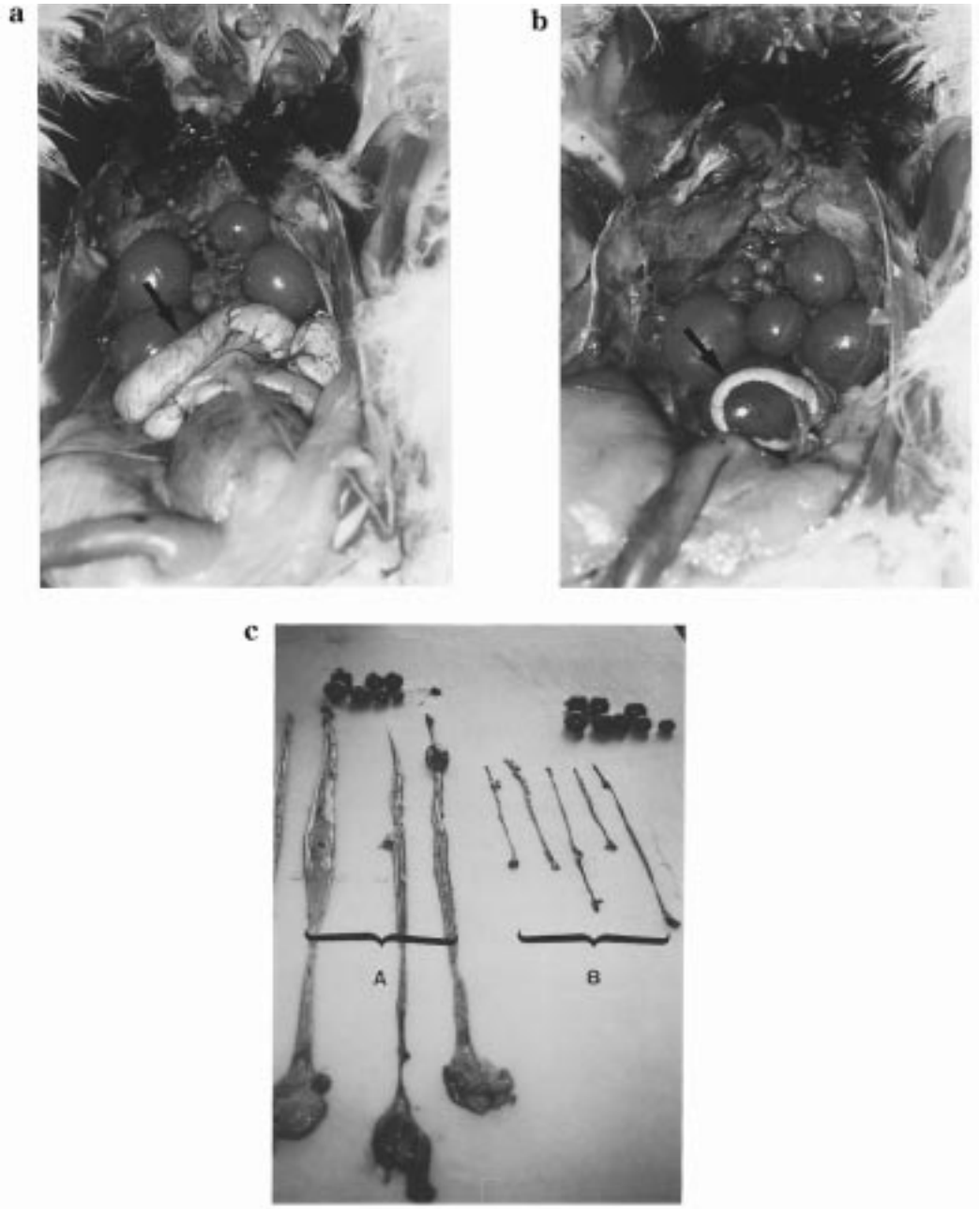

Figure 6. (a) Ovary with the array of follicular developments and the state of the oviduct development *in situ* in restricted birds, (b) ovary with the array of follicular developments and the state of the oviduct development *in situ* in jojoba supplemented birds; (c) ovary with the array of follicular developments and the length of four oviducts from four restricted birds (A) and five oviducts of five jojoba supplemented birds (B).

Table 2. Oviduct Weights (g per kg of BW) and Oviduct Lengths (cm) at Different Ages of Quantitatively Restricted Pullets (Restricted) and Pullets That Received a Jojoba-Supplemented Diet at 3−20 Weeks of Age[a]

		treatment		
age, weeks	oviduct	restricted	jojoba	
27	weight	18.57 ± 1.69	1.07 ± 0.12	*
35	weight	22.26 ± 1.09	1.62 ± 0.25	*
	length	70.60 ± 2.77	25.40 ± 1.89	*
60	weight	25.12 ± 3.94	0.83 ± 0.16	*
	length	62.40 ± 1.39	23.3 ± 2.20	*

[a] Asterisks denote significant overall treatment effect ($P < 0.001$).

at all in the oviducts of the jojoba-fed birds. They were significantly smaller in weight and shorter in length. The failure of oviposition was determined to be the failure of the oviduct's fimbrae to capture the ovulated follicle. This was largely due to the inadequate small size of the oviduct, resulting in internal lay. This virtual absence of the oviduct growth in the jojoba-fed birds demonstrates that jojoba meal contains factors which interfere with the normal development of the chicken oviduct.

The observation of some secondary sexual characteristics also showed that feeding jojoba meal inhibited comb and wattle growth in chickens. The imminent onset of sexual maturation in the C and R pullets was indicated externally by an accelerated rate of comb growth. This is usually due to increased levels of sex steroids in circulation, especially testosterone (Dorfman, 1962). Testosterone exerts its biological effect directly

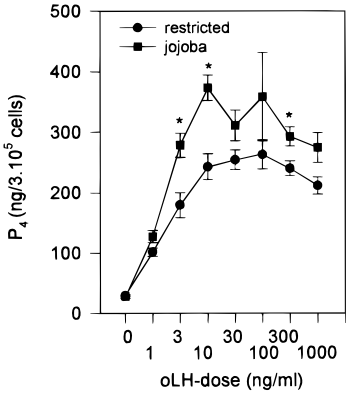

Figure 7. P_4 production *in vitro* of 3×10^5 granulosa cells from a large yellow follicle as a function of (R and JO) treatment and oLH doses. Values are expressed as means ± SEM. Asterisks indicate a significant difference between restricted and jojoba treatments at any oLH dose.

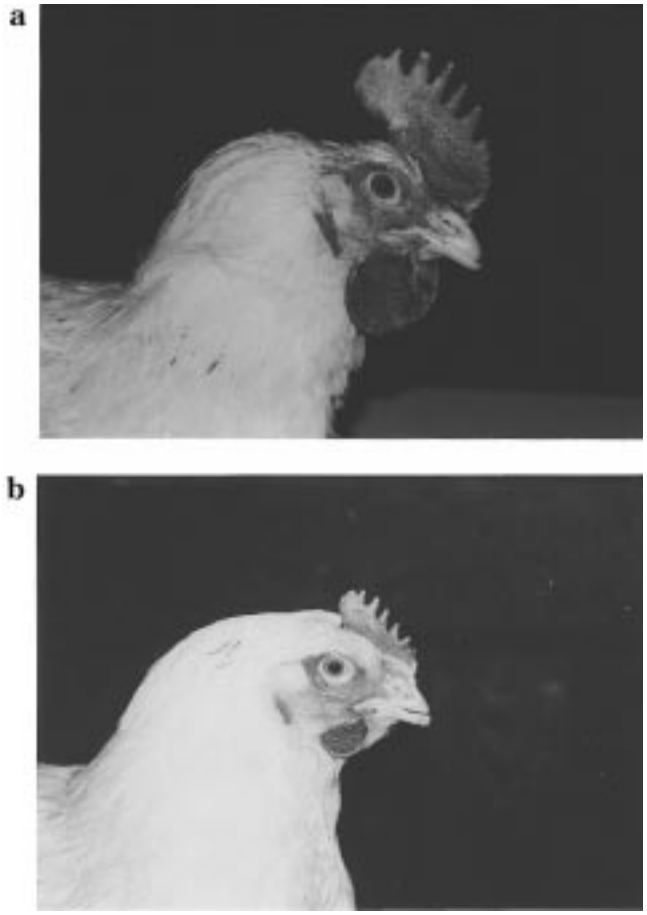

Figure 8. (a) Comb and wattle of a restricted bird; (b) comb and wattle of a jojoba-supplemented bird.

via the androgen receptors or via conversion to its metabolite dihydrotestosterone (DHT) or to E_2 (Swerdloff *et al.*, 1992). The reduced development of the combs and wattles suggests that testosterone may not be optimal in these pullets or probably that some components of jojoba interfere with the working mechanisms of endogenous steroids. Leitner *et al.* (1996) found that the administration of oestradiol-3-benzoate, a steroid antagonist, inhibited comb growth.

Taken together, these results point to some component of jojoba that is directly or indirectly inhibiting the development of the oviduct in the chickens fed jojoba meals. However, the nature of the inhibiting factor(s) or its mechanisms cannot yet be explained fully. We speculate that the increased levels of P_4 and T_3 resulting from feeding jojoba may play some contributing factor in the stunted development of the oviduct. It has been shown that oestrogens are mainly responsible for the development of the oviduct in immature pullets (O'Malley *et al.*, 1969). Similarly both P_4 and T_3 have been demonstrated to have roles in the process (Palmiter and Wrenn., 1971; Klandorf *et al.*, 1992). Also androgens are indispensable together with estrogens in the normal development of all oviduct segments (Mika *et al.*, 1987). After hatching, the oviduct is a small tubelike organ weighing about 10 mg. Under normal circumstances, under the influence of increasing levels of oestrogens, the epithelium of the oviduct starts to proliferate at the age of 8−10 weeks resulting in increased oviduct weight. Between 10 and 18 weeks of age, the weight increases rapidly due to lengthening of plicae and epithelium differentiation and mucosal and submucosal layers form folds to increase the diameter of the oviduct. During this period, oviductal cells are very sensitive and reactive to sex steroids. By the age of 18−20 weeks, a very pronounced increase in oviduct size is attained and gland formation and secretory proteins synthesis begin (Ylikomi and Tuohimaa, 1988).

It has been shown that E_2 indirectly stimulates the proliferation of oviduct epithelial cells *in vivo* (Dumas *et al.*, 1984) and that this indirect mechanism involves the cAMP regulatory systems. cAMP is involved in oestrogen target cell proliferation in the quail oviduct and cAMP phosphodiesterase (PDE) has an important regulatory function in the control of cell cycle progression. Thus an inhibition of this pathway could lead to the inhibition of oviduct growth. Such drugs as Tamoxifen have been used to inhibit growth through this pathway. The order of growth inhibition by Tamoxifen correlates with its PDE inhibitor activity. Indeed, Tamoxifen has been shown to inhibit basal and oestrogen-induced phospholipase A_2 activities in immature quail oviduct (Fayard *et al.*, 1992). It cannot be discounted that jojoba could contain such a factor which can act through this pathway to block oviduct development.

The undeveloped oviduct has also been shown to contain significant levels of P_4 receptors which are further increased during oestrogen-induced oviduct development (Boyd-Leinen *et al.*, 1984). At low doses, progesterone potentiates the action of oestrogen in stimulating the oviduct, but at higher levels, it is antagonistic (Palmiter and Wrenn, 1971). It is known that P_4 inhibits mitosis and tubular gland formation (Oka and Schimke, 1969). Since our data did not show any abnormality in the E_2 levels in the affected pullets but higher than normal P_4 during the steroid-sensitive period of oviduct development, we postulate that this massive level of P_4 may have antagonized the function of E_2 during this critical period. In explaining the possible causes of the failure of the development of the oviduct in jojoba-fed chickens, we cannot exclude the possibility that jojoba also contains a factor which can act directly on the oviduct to inhibit its growth. Maybe some factors can inhibit the synthesis of proteins that are necessary for proliferation, or maybe they are able to inhibit the synthesis of P_4, E_2, or testosterone receptors in the oviduct. It could also be factors which

block receptor sites for these steroids. Further studies are in progress to substantiate these.

Klandorf *et al.*(1992) showed that high levels of T_3/T_4, when injected into peripheral blood of 6−8-week-old broiler breeder pullets, can lead to reduced oviduct weights. This gives credence to our suggestion that the high T_3 in jojoba-fed pullets may be a contributory factor to the stunted development of their oviducts.

This inhibitory effect of jojoba meal on the oviduct development is specifically linked to poultry, since earlier studies in rats have demonstrated that the oviduct developed normally in weanling rats fed a jojoba-rich diet during rearing and gestation. Yet, an excess in fetal body weight reduction in these jojoba-supplemented rats was observed, due to a protein shortage during gestation. Moreover, in contrast with our observations in poultry, in rats this phenomenon was paralleled by decreased maternal plasma progestagens (Cokelaere *et al.*, 1993b).

In conclusion, in contrast with rats, reproduction in jojoba treated birds was impaired because of the absence of oviduct development. Our postulation is that this defect must be the result of some factors present in jojoba which either indirectly cause abnormally high levels of P_4 and T_3 and/or directly interfere with normal processes to inhibit oviduct development.

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