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Active Immunization of Japanese Quail Hens with a Recombinant Chicken Inhibin Fusion Protein Enhances Production Performance[1]

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ABSTRACT The effects of active immunization against inhibin on production performance in female Japanese quail (*Coturnix coturnix japonica*) were assessed in two separate trials using an MBP-cINA521 fusion protein as an immunogen. The fusion protein, MBP-cINA521, consisted of the bacterial maltose binding protein (MBP) and a truncated form of the mature α-subunit of chicken inhibin (cINA521). MBP-cINAl521 was constructed by: 1) excising a 521-bp *Pst*I fragment from a chicken inhibin α-subunit cDNA (cINA6; gift of P. A. Johnson), 2) cloning this fragment, which encodes all but the first 11 amino acid residues of the mature α-subunit, into the pMal-c2 vector of the MBP fusion expression system, and 3) expressing the fusion protein (MBP-cINA521) from the *Escherichia coli* and purifying it using affinity chromatography. In each trial, quail were randomly and equally assigned to one of two injection treatments as follows: 1) MBP-cINA521 in Freund's adjuvant, or 2) Freund's adjuvant (vehicular controls; CON). All immunizations were given subcutaneously and Freund's complete and incomplete adjuvant were used for primary and booster injections, respectively.

In Trial 1, birds were given a primary challenge of 0.2 mg MBP-cINA521 per bird at 25 d of age, followed by booster immunizations (0.1 mg MBP-cINA521 per bird) at 33, 40, 47, 54 and 61 d of age and every 35 d thereafter. The CON birds received vehicular immunizations at the same time intervals. In Trial 2, birds treated with MBP-cINA521 received a primary challenge of 0.2 mg MBP-cINA521 per bird at 26 d of age, followed by booster immunizations (0.1 mg MBP-cINA521 per bird) using the same schedule as that used in Trial 1, with the exception that no boosters were given after 61 d of age. The CON birds received vehicular immunizations at the same time intervals.

Collection of production performance data was initiated coincident with the laying of the first egg in each trial (i.e., beginning at 41 and 44 d of age for Trials 1 and 2, respectively) and continued for 30 1-wk periods of lay. Combined data from Trials 1 and 2 indicated that the mean SE age at first egg lay was markedly decreased ($P < 0.005$) in MBP-cINA521-treated quail (53.4 0.9 d of age) when compared to the CON (57.6 1.3 d of age). Likewise, the mean SE age at 50% egg production was reduced ($P < 0.03$) in quail immunized against inhibin (65.4 2.1 d of age) when compared to the CON (77.6 4.7 d of age). Total hen-day egg production was also higher ($P < 0.05$, Trial 1; $P < 0.01$, Trial 2) in MBP-cINA521-treated quail (88.7 1.4%, Trial 1; 90.1 1.2%, Trial 2) than in the CON birds (81.9 2.9%, Trial 1; 73.6 6.5%, Trial 2). Collectively, these findings provide evidence that inhibin immunoneutralization accelerated puberty and enhanced hen-day egg production during a 30-wk period of egg lay in Japanese quail.

(*Key words*: Japanese quail, inhibin, immunoneutralization, laying)

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INTRODUCTION

The existence of the hormone inhibin was first postulated more than 60 yr ago (McCullagh, 1932), yet its chemical isolation was only recently achieved (Miyamoto *et al*., 1985; Robertson *et al*., 1985). Inhibin and activin are dimeric glycoproteins that share a common α-subunit (Chen, 1993). Inhibin is comprised of

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Abbreviation Key: cINA521 = a truncated form of the mature α-subunit of chicken inhibin; CON = control; FSH = follicle-stimulating hormone; HDEP = hen-day egg production; Histag-cINA521 = vector encoding the recombinant polyhistidine-tagged fusion protein; IPTG = isopropyl-B-D-thiogalactoside; MBP = maltose binding protein.

a specific α-subunit and one of two related β-subunits, whereas activin is a hetero- or homodimer of two β-subunits (Rivier *et al*., 1987; de Jong, 1988; Ying, 1988; Risbridger *et al*., 1990). Primarily produced by the gonads, these two peptide hormones (inhibin and activin) exert opposite physiological effects on the control of pituitary follicle-stimulating hormone (FSH) secretion. Inhibin appears to depress, whereas activin stimulates, FSH secretion from the pituitary in both the basal and stimulated states (Vale *et al*., 1988). Excellent reviews on the isolation, production, assay, and biological actions of mammalian inhibin are available (see Rivier *et al*., 1987; de Jong, 1988; Ying, 1988; Risbridger *et al*., 1990).

Inhibin functions as an inhibitory feedback regulator of pituitary secretion of FSH, however it appears to also function in an autocrine or paracrine manner (Woodruff *et al*., 1990; Findlay 1993) by acting as a competitive FSH receptor antagonist. Because FSH plays an essential role in the recruitment and subsequent development of ovarian follicles, a successful strategy to induce hyperovulation in mammalian species has been the development of methods to neutralize endogenous inhibin activity.

Immunoneutralization of inhibin has been associated with increased ovulation rates in many mammalian species, including cattle, sheep, pigs, and rats (Forage *et al*., 1987; Findlay *et al*., 1989; Rivier and Vale, 1989; Brown *et al*., 1990; King *et al*., 1990; Mizumachi *et al*., 1990; Wrathall *et al*., 1990; Glencross *et al*., 1992; Scanlon *et al*., 1993). A variety of inhibin-based antigens have been used as vaccines in these studies. Accelerated ovulation rates found in mammals vaccinated with antigenic inhibin preparations is likely a consequence of elevated plasma FSH levels (Culler and Negro-Vilar, 1988; Rivier *et al*., 1988; Vale *et al*., 1988; Martin *et al*., 1991), which leads to enhanced ovarian follicular development. Despite conflicting data on how levels of FSH fluctuated during the ovulatory cycle, in all cycling mammals studied, immunoneutralization of endogenous inhibin has generally enhanced ovarian follicular development and ovulation rate, regardless of the antigen used or the species challenged.

Although the control of follicular recruitment and development within the ovary of the hen is poorly understood, pituitary gonadotropin involvement has been proven (Bahr and Johnson, 1984; Johnson, 1986), and a role for inhibin in the control of avian gonadotropin secretion and follicular development is becoming increasingly apparent (see reviews of Johnson, 1993, 1997). Inhibin likely exerts parallel physiological roles in follicular recruitment and development in fowl to those documented in mammals. However, in birds, involvement of inhibin in the control of ovulation rate may (Akashiba *et al*., 1988; Tsonis *et al*., 1988; Johnson *et al*., 1993) or may not (Johnson *et al*., 1993; Wang and Johnson, 1993a) be through suppression of pituitary FSH secretion.

The effects of inhibin immunoneutralization on egg production in avian species await investigation. Such an assessment, however, has been made possible by the recent cloning and sequencing of a cDNA encoding the α-subunit of chicken inhibin by Wang and Johnson (1993b).

Herein, a cDNA of the mature α-subunit of chicken inhibin (from cINA6; gift of P. A. Johnson, Cornell University, Ithaca, NY 14953) was digested with *Pst*I to produce a truncated DNA fragment that codes for 102 of 113 amino acids of the mature α-subunit (521 bp; cINA$_{521}$). cINA$_{521}$ was then expressed as a fusion protein with the *Escherichia coli* maltose binding protein (MBP-cINA$_{521}$) and used as an immunogen in female Japanese quail to assess changes in reproductive performance. Plasma immunological responses of the MBP-cINA$_{521}$-treated birds vs control-treated birds were also compared. Coturnix were chosen for study because their reproductive physiology, from the standpoint of the ovulatory process, is remarkably similar to that of egg-type chickens. This similarity is one of the many reasons why Coturnix serve as the bird of choice (pilot model) for extrapolation of research findings to the larger domestic fowl (such as chickens and turkeys) (See reviews of Kovach, 1974; Mills and Faure, 1992; Aggrey and Cheng, 1994; Jones, 1996).

MATERIALS AND METHODS

Cloning and Expression of Recombinant MBP-cINA$_{521}$

The fusion protein, MBP-cINA$_{521}$, consisted of MBP and a truncated form of the mature α-subunit of chicken inhibin. It was constructed by excising from a chicken inhibin α-subunit cDNA (cINA6; gift of P. A. Johnson) a 521-bp *Pst*I fragment encoding all but the first 11 amino acid residues of the mature α-subunit (cINA$_{521}$) and cloning it into the pMal-c2 vector of the MBP fusion expression system.[3] *Escherichia coli* containing the recombinant plasmid were cultured at 37 C in LB broth containing 2 g/L glucose and ampicillin (50 μg/mL). Cultures were grown to an optical density of 0.5 at 600 nm. Expression of the MBP-cINA$_{521}$ fusion protein was induced by addition of isopropyl-B-D-thiogalactoside (IPTG) at a final concentration of 0.3 m*M*, which causes derepression of the bacterial *Tac* promoter. Following induction with IPTG, cells were incubated (37 C) for an additional 3 h and then harvested by centrifugation at 4,000 *g* for 20 min (supernatant discarded). Cells were then resuspended (50 mL/L culture) in column buffer (20 m*M* Tris-HCL, pH 7.4; 200 m*M* NaCl; 1 m*M* EDTA) and frozen overnight.

[3]New England Biolabs, Beverly, MA 01915.

Purification of Recombinant MBP-cINA521

Bacterial lysis was accomplished by sonication of the thawed cell suspension (see above) using a Sonifier 450.[4] Sonication was conducted on ice for 14 min in seven 2-min pulses. Cell debris was removed by centrifugation (9,000 *g* for 30 min). The supernatant (containing MBP-cINA521) was diluted (1:5) in column buffer and applied to an affinity column of amylose / agarose resin. Unbound proteins were washed from the resin overnight with column buffer. MBP-cINA521 fusion protein was subsequently eluted from the amylose / agarose resin with 10 m*M* maltose in column buffer. Eluted fractions (8 mL) were evaluated for protein content by measurement of optical density at 280 nm. Proteins in the peak fractions were characterized by fractionation by SDS-PAGE (Laemmli, 1970) and visualized using a Coomassie stain. The predicted molecular weight of the MBP-cINA521 fusion protein was approximately 56 kDa. Fractions containing the MBP-cINA521 fusion protein were pooled and the protein concentration was determined using the Coomassie Plus Protein Assay[5] with BSA as the protein standard.

Development of Histag-cINA521 Fusion Protein and ELISA Analysis of Plasma Antibody Titers

To gain evidence of inhibin immunoneutralization, plasma immunological responses of representative MBP-cINA521- vs control (CON)-treated birds were compared. In quail, blood sampling is a most stressful event. It invariably interferes with egg laying and can be lethal. Therefore, we chose not to blood sample the birds during laying in either trial (see Vaccination Treatments, below). Furthermore, because the Histag-cINA521-based ELISA (described below) was not developed until after the conclusion of Trial 1, we waited until the end of Trial 2 to collect plasma from subject animals. Although it was likely that titers to the immunogen would be diminished at the conclusion of this trial, particularly as booster injections were discontinued after 61 d of age, we reasoned that demonstration of even a small immunological response would give evidence of a mechanism of action and was, thus, an important part of our experiments.

We initially attempted to characterize the presence and specificity of the hen's humoral immune response to MBP-cINA521 using Factor Xa cut MBP-cINA521 on Western immunoblots. Although antibodies in the plasma of quail treated with the immunogen showed a positive and specific response to the inhibin fragment and MBP, we were unable to produce publication-quality results, even after repeated attempts (data not shown). We believe this is due to the fact that, because anti-quail secondary antibodies were not available commercially, we were compelled to use anti-chicken secondary antibodies, which probably have lower affinity to quail immunoglobulins. Still wishing to determine whether an immunological response to inhibin was generated by our MBP-cINA521 treatment, we decided to construct a solid phase immunoassay (ELISA). To do so, the 521-bp *Pst*I fragment from the cDNA encoding the α-subunit of chicken inhibin was cloned into the pTrcHis vector for the expression of a polyhistidine-tagged fusion protein.[6] The vector encoding the recombinant polyhistidine-tagged fusion protein (Histag-cINA521) was expressed in *E. coli* and Histag-cINA521 was isolated using immobilized metal affinity chromatography under denaturing conditions (8 *M* urea). The isolated fusion protein was characterized by SDS-PAGE and protein concentration determined using the Coomassie Plus Protein Assay (data not shown). The Histag-cINA521 fusion protein was then used to develop our ELISA (see below) for testing for the presence of antibody titers directed specifically against the truncated α-subunit of chicken inhibin (cINA521) and not the entire fusion protein (MBP-cINA521). The latter contains the highly antigenic bacterial MBP.

Our ELISA was a modification of a protocol previous described by Rejman *et al.* (1989). Specifically, the Histag-cINA521 fusion protein was the antigen coated onto each well of a 96-well microtiter plate (250 μg per well in carbonate buffer, pH 9.6). All wells were blocked for nonspecific binding with 1 PBS (140 m*M* NaCl, 1.5 m*M* KH_2PO_4, 8.0 m*M* Na_2HPO-7 H_20, 2.7 m*M* KCl; pH = 7.4) / Tween-20 (0.05%) + 1% BSA for 1 h at room temperature. At the end of the study, representative birds (n = 5) from each group (CON- and MBP-cINA521-treated) were bled and plasma were obtained following centrifugation. Plasma antibody samples were serially diluted to 1:250, 1: 500, 1:1,000, 1:2,000, 1:4,000, and 1:8,000 in PBS-Tween-20 + 1% BSA. Each dilution in duplicate was applied into wells coated with the Histag-cINA521 fusion protein and incubated for 2 h at room temperature. Alkaline phosphatase conjugated rabbit anti-chicken IgG[7] diluted 1:5,000 was added to each well and incubated for 1 h at room temperature. The soluble substrate *p*-nitrophenyl phosphate[7] was added into each well and color allowed to develop for 45 min. The optical density of each well at 405 nm was measured using the SLT Rainbow Microplate Reader.[8]

Animals and Husbandry

Female Japanese quail (*Coturnix coturnix japonica*) hatched from a randombred line of birds maintained at the Louisiana State University Department of Poultry Science were used in the present study. In each trial (see Vaccination Treatments, below), approximately 150 chicks were removed from the incubator on Day 17 of incubation,

[4]Branson Ultrasonics Co., Danbury, CT 06810.
[5]Pierce, Rockford, IL 61105.
[6]Xpress System, Invitrogen Co., Carlsbad, CA 92008.
[7]Sigma-Aldrich, St. Louis, MO 63160.
[8]Tecan US Inc., Research Triangle Park, NC 27709.

leg-banded, and transferred in mixed-sex groups of 50 to each of three decks of a Petersime 2S-D brooder battery[9] modified for quail. Initial brooding temperature was 37.8 C with a weekly temperature decline of 2.8 C until an ambient temperature of 23.9 to 26.7 C was achieved. A quail starter ration (28% CP, 2,800 kcal ME/kg) and water were supplied for *ad libitum* consumption throughout the growing period (until 6 wk of age). Adults consumed a laying ration (21% CP, 2,750 kcal ME/kg) and water *ad libitum*. During the brooding period, birds were housed under continuous dim light (22 lx). At 5 wk of age, a 14 h light (280 to 300 lx): 10 h dark cycle was used to stimulate egg lay in adult quail. Lights-on occurred at 0500 h; daily maintenance and feeding chores were done at the same time each day (0900 h).

Vaccination Treatments

At 25 and 26 d of age (Trials 1 and 2, respectively), birds were wing-banded and sexed. Females were subsequently housed in individual laying cages by equal and random assignment to one of two injection treatments (Trial 1, 25 birds per treatment; Trial 2; 24 birds per treatment) as follows: 1) MBP-cINA521 in Freund's adjuvant, or 2) Freund's adjuvant (vehicular controls; CON). All injections (1.0 mL, primary injections; 0.5 mL, boosters) were given subcutaneously along the dorsal midline at the level of the scapulae and Freund's complete and incomplete adjuvant were used for primary and booster injections, respectively (see below). Less than 2% of the birds developed Freund's-related reactions at the injection site.

In Trial 1, MBP-cINA521-treated birds were given a primary immunization (0.2 mg MBP-cINA521 per bird) at 25 d of age, followed by booster injections (0.1 mg MBP-cINA521 per bird) at 33, 40, 47, 54 and 61 d of age and every 35 d thereafter. The CON birds received vehicular injections at these same time intervals. In Trial 2, MBP-cINA521-treated birds received a primary immunization (0.2 mg MBP-cINA521 per bird) at 26 d of age, followed by booster injections of equivalent concentration and times of administration as used in Trial 1, with the exception that no boosters were given after 61 d of age. The CON birds received vehicular injections at these same time intervals.

Variables Measured and Statistical Analyses

Collection of production performance data was initiated coincident with the laying of the first egg in each trial (i.e., beginning at 41 and 44 d of age for Trials 1 and 2, respectively) and continued for 30 1-wk periods of lay. Daily hen-day egg production (HDEP) and mortality measures were recorded during this time. In addition, average age at first egg and age at which hens reached 50% egg lay were calculated for each injection treatment group (CON vs MBP-cINA521) within a trial.

[9]Petersime Incubator Co., Gettysburg, OH 45328.

Within a trial, HDEP data were subjected to a one-way ANOVA which incorporated a completely randomized design with a split-plot arrangement of treatments. The main plot consisted of the two injection treatments (CON vs MBP-cINA521) and the 30 laying periods of 7 d each comprised the split. In addition, average age at first egg lay and age at which hens reached a rate of 50% egg production were calculated for each treatment group. Injection treatment differences in these two indices of puberty were assessed by a two-way ANOVA using a randomized block design RBD in which trials were considered as blocks (replications). For the ELISA data, injection treatment differences were detected at each plasma serial dilution tested using a one-way ANOVA within a completely randomized design.

RESULTS

Production and Characterization of Recombinant MBP-cINA521

The SDS-PAGE analyses of the expressed and purified MBP-cINA521 are shown in Figure 1 (only column fractions 1, 5, 8, 11, 14, 16, and 31 were chosen for illustrative purposes). The MBP-cINA521 fusion protein, as identified by its apparent molecular weight of 56.6 kDa, was present in several fractions. Examples of protein peaks are represented by fractions 11, 14, and 16. As expected, the fusion protein could not be detected in early (e.g., prepeak fractions 1, 5, and 8) or late (e.g., postpeak fraction 31) eluants. The SDS-PAGE analysis of pooled peak protein fractions treated with Factor Xa protease identified two additional protein bands with the approximate molecular weights of 43.8 and 12.8 kDa, corresponding to the apparent molecular weights of MBP and the α-subunit fragment of chicken inhibin, respectively. Traces of the entire MBP-cINA521 fusion protein was also identified, presumably due to incomplete proteolytic digestion. Extraneous proteins present in eluted and pooled fractions are likely bacterial protein contaminants present in low concentrations (Figure 1).

Immune Responses Following Active Immunization Against MBP-cINA521

Histag-cINA521 was expressed in *E. coli* and purified under denaturing conditions using affinity chromatography. The SDS-PAGE characterization of the purified fractions showed a single prominent band with an apparent molecular weight of 15.4 kDa, in keeping with the predicted molecular weight of the fusion protein (data not shown). The ELISA analyses demonstrated the presence of antibodies directed against the recombinant inhibin fragment (Histag-cINA521) in the plasma of representative MBP-cINA521-challenged birds but not in CON plasmas (Table 1). Significant injection treatment differences (range: $P < 0.0005$ to $P < 0.02$) existed at all plasma serial dilutions tested.

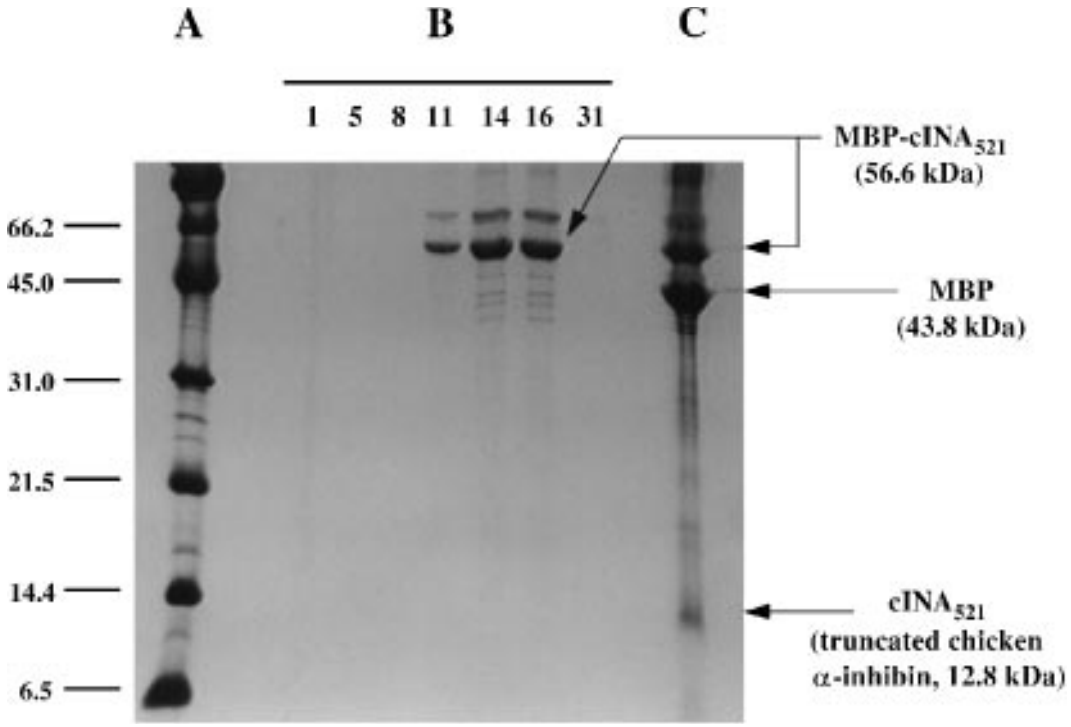

FIGURE 1. SDS-PAGE analysis of recombinant MBP-cINA521. Lane A: molecular weight markers [bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), bovine carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), apotinin (6.5 kDa)]; Lane B: representative elution fractions of recombinant MBP-cINA521 from affinity column (fraction number indicated); Lane C: recombinant MBP-cINA521 treated with Factor Xa protease, inhibin fragment (cINA521), MBP, and undigested fusion protein (MBP-cINA521) are indicated by arrows.

Production Performance Following Immunoneutralization of MBP-cINA521

Although egg and shell quality measures (e.g., misshapen, checked, soft-shelled, and double-yolked eggs; egg weight, and specific gravity) were not collected, our subjective impression was that such variables were not affected by treatment with MBP-cINA521. Livability was also similar (data not shown) between the two treatment groups in both trials.

The combined results of puberty data from Trials 1 and 2 showed that mean age at first egg lay and age at 50% egg production were dramatically decreased ($P < 0.005$ and $P < 0.03$, respectively) in immunized quail (Table 2). Furthermore, total HDEP (Table 3; Figure 2) was higher ($P < 0.05$, Trial 1; $P < 0.01$, Trial 2) in MBP-cINA521-treated quail than in the CON birds.

DISCUSSION

A fusion protein, MBP-cINA521, consisting of the bacterial maltose binding protein (MBP) and a truncated form of the mature α-subunit of chicken inhibin (cINA521) was constructed. This construction was accomplished by: 1) excising a 521-bp *PstI* fragment from a chicken inhibin α-subunit cDNA, a digest fragment that encoded all but the first 11 amino acid residues of the mature α-subunit, 2) cloning this fragment into the pMal-c2 vector of the MBP fusion expression system, and 3) expressing, from *E. coli*, the fusion protein, MBP-cINA521. The latter was purified using immunoaffinity column chromatography and characterized using SDS-PAGE.

Electrophoretic results demonstrated that the MBP-cINA521 fusion had a molecular weight of approximately

TABLE 1. Mean SE optical density measurements from ELISA analysis of antibodies directed against recombinant Histag-cINA521 in serially diluted plasma taken from quail hens injected with either the vehicular control (CON) or actively immunized against inhibin using MBP-cINA521[1]

		Plasma dilution											
Treatment[2]	N	1:250		1:500		1:1,000		1:2,000		1:4,000		1:8,000	
CON	5	0.139	0.029	0.101	0.018	0.082	0.011	0.061	0.006	0.051	0.004	0.039	0.002
MBP-cINA$_{521}$	5	0.461	0.049	0.309	0.047	0.214	0.040	0.137	0.026	0.093	0.012	0.063	0.006

[1]Histag-cINA521 = vector encoding the recombinant polyhistidine-tagged fusion protein. MBP-cINA521 = maltose-binding protein with a truncated form of the mature α-subunit of chicken inhibin.

[2]Within each plasma dilution (columns), significant treatment differences exist (range in P values: < 0.0005 to 0.02).

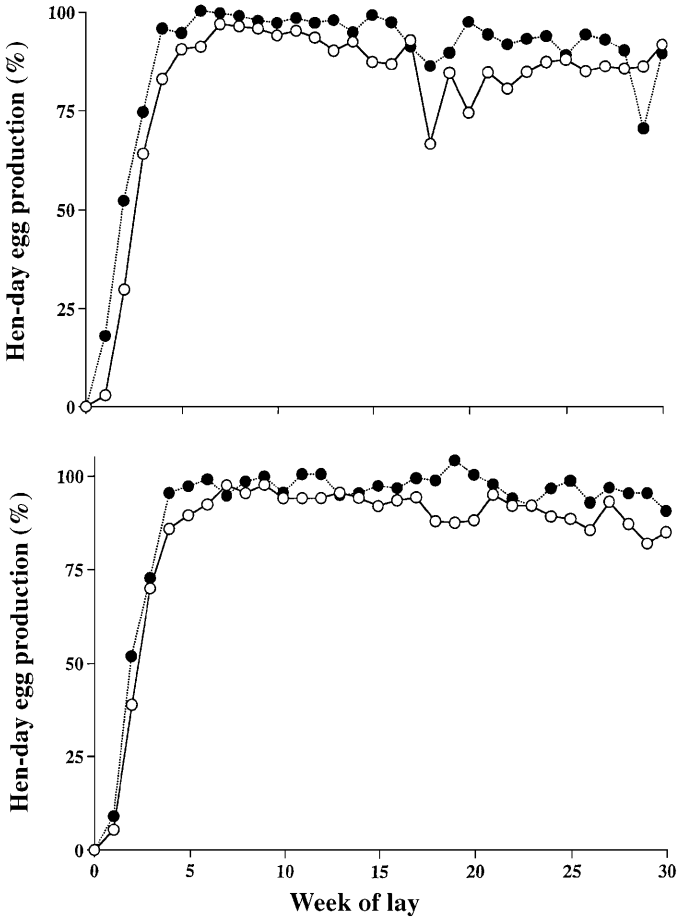

FIGURE 2. Mean weekly hen-day egg production (HDEP) of Trials 1 and 2. Percentage HDEP in quail hens actively immunized against inhibin using MBP-cINA521 (●) is compared to hens injected with the vehicular control (o) from Trial 1 (upper graph) and Trial 2 (lower graph).

56.6 kDa. Factor Xa cleavage of MBP-cINA521 resulted in two distinct proteins. Estimation of their molecular weights by SDS-PAGE was consistent with an interpretation that the heavier protein (approximately 43.8 kDa) was MBP and the lighter protein (approximately 12.8 kDa) was the truncated chicken α-inhibin fragment, cINA521. The molecular weight for MBP is 42.7 kDa. The apparent molecular weight of cINA521 is in agreement with the molecular weight reported for the mature α-subunit (13.6 kDa; Wang and Johnson, 1993b), when corrected for the loss in molecular weight that would be attributed to the elimination of the first 11 amino acid residues of the chicken α-subunit of inhibin.

Immunoneutralization of inhibin has been associated with increased ovulation rates in heifers (Glencross *et al*., 1992; Scanlon *et al*., 1993), sheep (Forage *et al*., 1987; Findlay *et al*., 1989; Mizumachi *et al*., 1990; Wrathall *et al*., 1990), gilts (Brown *et al*., 1990; King *et al*., 1990), and rats (Rivier and Vale, 1989). Some of the antigens tested in these studies included: recombinant DNA derived inhibin α-subunit, synthetic peptide fragments of inhibin α-subunit, and partially purified inhibin from follicular fluid.

Herein, active immunization of quail using MBP-cINA521 resulted in a marked reduction of the mean ages at first egg lay and 50% egg production and it increased total HDEP during a 30-wk period of lay in two separate trials. To our knowledge, this is the first report that immunoneutralization of inhibin positively impacts female reproductive performance in an avian species. Our production performance findings in the quail are consistent with similar reports in mammals (see above) that inhibin immunoneutralization has a positive impact on ovulation rate.

Our studies were not designed to determine the mode(s) of action by which active immunization against inhibin influences egg lay in birds. Nevertheless, previous studies suggest that inhibin acts at the pituitary to suppress FSH secretion and may have a paracrine function in the gonads by competing for FSH receptor binding sites (Woodruff *et al*., 1990; Findlay, 1993). Therefore, in mammals, and perhaps also in birds, accelerated ovulation rates postvaccination with antigenic inhibin preparations can be explained as the logical end result of elevated plasma FSH levels (Culler and Negro-Vilar, 1988; Rivier *et al*., 1988; Vale *et al*., 1988; Martin *et al*., 1991) or greater availability of

TABLE 2. Mean SE ages at first egg lay and at 50% egg production in quail injected with either the vehicular control (CON) or actively immunized against inhibin using MBP-cINA521 (combined data, Trials 1 and 2)[1]

Treatment	Age at first egg		Age at 50% egg production	
	(d)			
CON	57.6	1.3[A]	77.6	4.7[a]
MBP-cINA521	53.4	0.9[B]	65.4	2.1[b]

[a,b]Means within a column with no common superscript differ significantly ($P < 0.03$).

[A,B]Means within a column with no common superscript differ significantly ($P < 0.005$).

[1]MBP-cINA521 = maltose-binding protein with a truncated form of the mature α-subunit of chicken inhibin.

TABLE 3. Mean SE hen-day egg production (HDEP) during a 30-wk period of lay in quail injected with either the vehicular control (CON) or actively immunized against inhibin using MBP-cINA521[1]

Trial	n	HDEP			
		CON		MBP-cINA521	
		(%)			
1	25	81.9	2.9[a]	88.7	1.4[b]
2	24	73.6	6.5[A]	90.1	1.2[B]

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within a row with no common superscript differ significantly ($P < 0.01$).

[1]MBP-cINA521 = maltose-binding protein with a truncated form of the mature α-subunit of chicken inhibin.

ovarian FSH receptors for binding with FSH. Either or both of these conditions could lead to enhanced ovarian follicular recruitment and development. It should be noted that the inhibin-FSH relationship is not always straightforward. However, despite conflicting data on how levels of FSH fluctuated during the ovulatory cycle, immunoneutralization of endogenous inhibin has generally enhanced ovarian follicular development and ovulation rate, in all mammals studied, regardless of the antigen used or the species challenged.

The demonstration of a humoral immune response of MBP-cINA521-treated birds to Histag-cINA521 is also in keeping with the proposed mechanism of MBP-cINA521 active immunization as it relates to production performance. The presence of antibodies that recognized the Histag-based recombinant inhibin in the plasma of MBP-cINA521-challenged quail suggests that vaccination with MBP-cINA521 produced an immunoneutralization of inhibin's endocrine or paracrine actions relative to FSH. With the diminution of the negative influences of inhibin on FSH, we suggest that maturation of eggs in the follicular hierarchy was enhanced, which would explain our observations of an early onset of puberty and increased HDEP rates in birds vaccinated with MBP-cINA521.

In conclusion, these studies demonstrated that active immunization against inhibin in Japanese quail enhanced production performance. Japanese quail have been selected for intensity of egg lay and serve as a useful model for egg laying domestic fowl. Our ability to enhance production performance in a species previously selected for intensity of egg laying suggests that immunization against inhibin in domestic fowl could produce similar results, particularly in the broiler breeder, but perhaps also in the Single Comb White Leghorn. Further studies of inhibin immunoneutralization will be required to determine the precise mechanism of action in avians as well as its usefulness to the poultry industry.

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