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DESENVOLVIMENTO ÓSSEO DA PROGÊNIE DE REPRODUTORAS PESADAS SUPLEMENTADAS COM FONTES INORGÂNICAS E ORGÂNICAS DE ZINCO, MANGANÊS E COBRE

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DESENVOLVIMENTO ÓSSEO DA PROGÊNIE DE REPRODUTORAS PESADAS SUPLEMENTADAS COM FONTES INORGÂNICAS E ORGÂNICAS DE ZINCO, MANGANÊS E COBRE[1]

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RESUMO

A presente tese foi realizada com o objetivo de avaliar os efeitos da nutrição mineral materna no desenvolvimento esquelético da progênie. Desta forma, um estudo foi conduzido com reprodutoras pesadas suplementadas com zinco (Zn), manganês (Mn) e cobre (Cu) inorgânico e orgânico. Os tratamentos foram fornecidos para as reprodutoras pesadas Cobb 500 de 22 a 68 semanas de idade (em ppm de Zn, Mn e Cu, respectivamente): 100, 100 e 10 com fontes de sulfato (IZMC, Controle); uma combinação de 60, 60 e 3 na forma de sulfato mais 40, 40 e 7 na forma de aminoácido complexado (OZMC; ISO); e o tratamento Controle mais 40, 40 e 7 de OZMC (ON TOP). Cada tratamento possuía 10 repetições de 20 fêmeas e 2 machos cada. Os tratamentos não influenciaram a taxa de postura e a produção de ovos por ave alojada, entretanto, aves alimentadas com OZMC na forma ISO ou ON TOP aumentaram peso e espessura da casca do ovo comparado ao tratamento Controle. As fontes de minerais fornecidas à geração parenteral influenciou na composição do ovo, assim como, no desenvolvimento esquelético da progênie. A suplementação de microminerais orgânicos na forma ISO aumentaram a concentração de Zn no albúmen e gema comparado ao tratamento Controle, entretanto, a mesma tendência não ocorreu com Mn e Cu. Aos 18 dias de incubação (E18) os embriões oriundos de matrizes que receberam os tratamentos ISO e ON TOP aumentaram a espessura de tíbia em 2% comparado ao Controle, indeferentemente da idade da matriz. No mesmo sentido, aos E18 a tíbia e fêmur apresentaram uma maior calcificação para as aves que receberam o tratamento Controle mais a suplementação de OZMC ON TOP. Ao nascimento, o momento de inércia (indicador de resistência e rigidez óssea) das tíbias foram superiores para os tratamentos que receberam a suplementação de OZMC ISO ou ON TOP. Conclui-se que a suplementação adicional de minerais orgânicos em conjunto com fontes inorgânicas comumente utilizadas de Zn, Mn e Cu melhoram a qualidade da casca e o desenvolvimento esquelético dos embriões ao nascimento.

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[1]Tese de Doutorado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (166 p.) Fevereiro, 2013.

PROGENY BONE DEVELOPMENT FROM BREEDER HENS FED DIETS SUPPLEMENTED WITH ZINC, MANGANESE AND COPPER FROM INORGANIC AND ORGANIC SOURCES[2]

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ABSTRACT

This thesis was carried out to evaluate the effects of maternal nutrition on progeny skeletal development. Thus, a study was conducted with breeder hen diets supplemented with zinc (Zn), manganese (Mn), and copper (Cu) from inorganic and organic sources. The treatments were provided to Cobb 500 breeder hens from 22 to 68 wk of age (in ppm of Zn, Mn and Cu, respectively): 100, 100 and 10 from sulfate (IZMC; Control); a mixture of 60, 60, and 3 from sulfate plus 40, 40, and 7 from amino acid-complex (OZMC) combination (ISO), and the Control treatment plus 40, 40 and 7 from OZMC (ON TOP). Each treatment had 10 replications of 20 females and 2 males. Breeder productions such hen day egg production and eggs per hen housed were not affected by the treatments, however, hens fed OZMC supplementation as ISO or ON TOP increased eggshell weight and thickness compared to the Control diet. The minerals source provided to the parenteral generation influenced the egg composition, as well as the progeny skeletal development. Feeding ISO treatment compared to the Control diet increased the Zn content of the yolk and albumen blend, however, the same effect was not observed for Mn and Cu. Embryos at 18 days of incubation (E18) from hens fed ISO and ON TOP diets had 2% thicker tibia compared to the Control, regardless of hen age. In the same way, at E18 the tibia and femur calcification were greater from hens fed the Control treatment plus ON TOP OZMC supplementation. At day of hatch the chicks from groups fed ISO or ON TOP OZMC increased the tibia moment of inertia (indicator of bone strength and stiffness). It is concluded that the additional supplementation of OZMC together with IZMC can improve the eggshell quality and the embryo bone development.

[2]Doctoral thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (166 p.) February, 2013.

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RELAÇÃO DE ABREVIATURAS E SÍMBOLOS

Cd	Cádmio
Ca	Cálcio
Cl	Cloro
Co	Cobalto
Cu	Cobre
S	Enxofre
Fe	Ferro
F	Flúor
P	Fósforo
CBH	Hipersensibilidade cutânea basófila
Mn	Manganês
Mg	Magnésio
Hg	Mercúrio
Mo	Molibdênio
K	Potássio
Se	Selênio
Na	Sódio
Zinco	Zn

CAPÍTULO I

Introdução

Uma das metas que se busca na nutrição de reprodutoras pesadas é alimentar as aves para maximizar a produção de ovos, assim como, o desempenho da progênie. Neste contexto, os nutrientes transferidos da reprodutora para o ovo durante sua formação é de fundamental importância para o adequado crescimento e desenvolvimento do embrião. O conhecimento de como a nutrição de reprodutoras, influencia o desenvolvimento fisiológico do embrião, pode justificar a inclusão de determinados ingredientes na formulação de ração com o intuito de melhora da qualidade de pintos de um dia de idade. Sabe-se que, a taxa de eclosão de ovos em empresas de grande porte, reconhecidas pela sua excelência no processo de produção de pintos de um dia dificilmente excede 87% (Leeson & Summers, 2001). Desta forma, existe um grande caminho a ser percorrido até que os restantes 13% dos ovos sejam convertidos em pintinhos, maximizando a eficiência do processo e reduzindo custos. A produção anual de ovos destinados a produção de pintos de corte no Brasil é de mais de 7 bilhões (UBA, 2011), sendo assim, qualquer melhora na eclodibilidade destes ovos resultaria em um aumento significativo no número de pintinhos.

Microminerais como zinco (Zn), manganês (Mn) e cobre (Cu) são componentes do ovo, essenciais para o adequado crescimento e desenvolvimento do embrião. Estes minerais estão envolvidos em uma série de processos bioquímicos essenciais, dentre eles a formação do tecido ósseo. Além disso, há indicativos de que alterações nos membros locomotores de frangos de corte podem estar associadas a desordens metabólicas iniciadas ainda durante o período embrionário (Dibner et a., 2007).

A suplementação de Zn, Mn e Cu em dietas para reprodutoras pesadas geralmente é feita a partir de sais inorgânicos como óxidos e sulfatos, entretanto, o uso de microminerais na forma orgânica recentemente têm mostrado efeitos positivos no desempenho e imunidade da progênie. Há evidências de que o Zn, Mn e o Cu ligados a aminoácidos são mais biodisponíveis em comparação as fontes inorgânicas tradicionais (Wedekind et al., 1992). Alguns trabalhos foram realizados procurando avaliar a imunidade de pintinhos provenientes de reprodutoras recebendo diferentes fontes de microminerais (Kidd et al., 2000; Hudson et al., 2004ab), porém a busca pelo conhecimento envolvendo a nutrição das reprodutoras pesadas e o desenvolvimento esquelético da progênie, representam uma grande oportunidade de desenvolvimento e investigação científica.

Com base na carência de informações sobre a nutrição mineral do embrião durante a incubação e a relevância deste período para o crescimento de frangos de corte, a presente tese foi conduzida a fim de avaliar os efeitos da suplementação de Zn, Mn e Cu complexados a aminoácidos, em dietas para reprodutoras pesadas, sobre o desenvolvimento embrionário dos tecidos de sustentação como ossos e cartilagens.

Revisão bibliográfica

Utilização de microminerais na avicultura

Historicamente na indústria avícola, os microminerais têm sido suplementados nas dietas utilizando fontes salinas, as quais geralmente apresentam um baixo custo. Estas fontes, normalmente não são submetidas a uma avaliação técnica quanto à sua qualidade, especialmente por serem necessárias em uma pequena quantidade na ração e estar presente nos demais componentes da dieta.

A disponibilidade variável, assim como, a presença de contaminantes, são fatores importantes que devem ser considerados quando microminerais são suplementados. O óxido de Zn e o sulfato de Cu, por exemplo, são comumente utilizados na alimentação animal, entretanto, são muitas vezes fontes derivadas de resíduos da indústria siderúrgica, que pode potencialmente apresentar elevados níveis de contaminantes, tais como flúor (F) e cádmio (Cd) (Vieira, 2008). Regulamentações de mercado, como os emitidos pela União Européia (CEC, 1999), levantou novas preocupações para o mercado de produção de carnes, com várias limitações em termos de metais pesados e outros contaminantes em alimentos para animais de produção.

A determinação das exigências de microminerais, têm sido uma preocupação secundária na avicultura em comparação com outros nutrientes (Vieira, 2008). A solubilidade da fonte utilizada, bem como, o status nutricional dos animais, são fatores muito importantes na determinação da exigência de minerais. Pesquisas para determinar a exigência mineral das aves deve levar em consideração sua função fisiológica, nos respectivos sistemas biológicos. Estas funções, podem ser geralmente divididas em contrução e manutenção de tecidos duros e moles, assim como, na regulação de processos biológicos onde os minerais essenciais atuam como catalisadores enzimáticos, regulam sistemas hormonais, e também como parte integrante e ativadora de metaloenzimas (Underwood, 1977).

A absorção intestinal dos minerais está sempre sob interferências que afetam a utilização dos mesmos, por isso, é essencial minimiza-las. Há diversos fatores antagônicos conhecidos, que causam variações nas taxas e níveis de absorção sob diferentes condições entéricas. Podem ser formados precipitados insolúveis por várias causas, como a disputa pelo mesmo ligante, que pode ser orgânico como o ácido fítico, ou inorgânico como o fosfato; ambos podem reduzir ou inibir completamente a disponibilidade do mineral (Cabell & Earle, 1965; Vohra et al., 1965). Os fitatos reduzem a absorção de Zn (Hempe & Cousins, 1989) e o cálcio (Ca) diminui a absorção de Zn e Cu (Lowel et al., 1994; Wedekind et al., 1994). O Cu e o molibdênio (Mo) são minerais fortemente antagônicos, enquanto Mn e ferro (Fe) competem por mecanismos de absorção semelhantes (Sandstrom 1992).

O processo digestivo pode aumentar ou restringir as proporções de minerais ingeridos que são absorvidos a partir da dieta, e ocasionalmente, mudar as formas em que são absoridas (ex, selênio (Se) e enxofre (S). No entanto, os minerais não são digeridos (quebrados) em formas metabolizadas como ocorre com componentes orgânicos da dieta. A absorção dos minerias ocorre predominantemente no duodeno das aves (Ashmead, 1993). A taxa de absorção é depende de fatores como pH, carreadores, forma química e física dos minerais e a presença de outros minerais que podem influenciar a absorção dos mesmos (Hill & Matrone, 1970). Vários mecanismos de absorção de minerais têm sido elucidados, com raras exceções, a absorção de minerais depende da capacidade dos elementos ligar-se à proteína transportadora presente na membrana do enterócito. Após esta ligação, os minerais são transportados para o citoplasma da célula através de difusão passiva ou transporte ativo. Muitos minerais como Zn, Fe e sódio (Na), necessitam de um sistema de transporte ativo onde sua absorção é cuidadosamente regulada (Garrick et al., 2003; Bai et al., 2008). Os minerais após absorvidos pela mucosa intestinal são transportados para o fígado na forma livre ou ligada, através do sistema porta-hepático. Do fígado, eles são transportados pela corrente sanguínea periférica até os diferentes órgãos e tecidos, as taxas são determinadas por mecanismos de transportadores locais na membrana celular e organelas intracelulares.

Os principais sais inorgânicos utilizados em avicultura para suplementar Zn, Mn e Cu são fontes de sulfatos ($ZnSO_4$, $MnSO_4$, e $CuSO_4$) e óxidos (ZnO, MnO, e CuO). Além disso, estas fontes inorgânicas foram utilizadas para desenvolver as exigências nutricionais das aves (NRC, 1994). O uso de sais inorgâncios pode resultar em baixa biodisponibilidade do mineral para os animais, primeiramente devido a um grande número de antagonismos presentes entre nutrientes e ingredientes, o qual prejudica a absorção (Underwood e Suttle, 2001). Talvez o antagonismo mais relevante na nutrição mineral de aves seja entre os minerais divalentes como Zn, Mn, Cu e o fitato. O fitato é capaz de formar quelatos com estes minerais bastante estável e altamente insolúvel (Leeson e Summers, 2001). O antagonismo é bastante forte onde por exemplo, a ligação de Ca, Zn e outros minerais pode reduzir a disponibilidade de fósforo (P), mesmo na presença de uma fitase exógena (Tamim e Angel, 2003). Antagonimos também podem ocorrer entre um mineral e outro, como ocorre com níveis elevados de Zn que podem reduzir a disponibilidade de Cu (Evans et al., 1975). A disponibilidade de fontes inorgânicas de minerais também podem ser reduzidas por outros nutrientes como, por exemplo, a disponibilidade de Cu pode ser reduzida pelo ácido ascórbico (Carlton e Henderson, 1965). O que existe em comum nestas interações parece ser a dissociação dos sais inorgânicos em um pH relativamente baixo do sistema gastrointestinal superior (próventrículo e moela). Quando o mineral atinge um pH mais elevado nos segmentos posteriores (intestino), o mesmo ioniza o metal que pode ligar-se a uma série de outros minerais, nutrientes e componentes não nutritivos da digesta formando um quelato insolúvel, indigestível e portanto excretado.

Fontes orgânicas ou quelatos de minerais tem sido avaliadas devido a sua perspectiva de serem mais biodisponíveis que na forma inorgânica (Kidd, 2003; Dibner et al., 2007). Os minerais quelatados são definidos por Leeson & Summers (2001), como sendo uma mistura de elementos minerais que são ligados a algum tipo de carreador, o qual pode ser um aminoácido ou polissacarídeo, que possui a capacidade de se ligar ao metal por ligações covalentes, através de grupamentos aminos ou oxigênio, formando assim uma estrutura cíclica.

São denominados quelatos compostos formados por íons metálicos seqüestrados por aminoácidos, peptídeos ou complexos polissacarídeos que proporcionam a esses íons alta disponibilidade biológica, alta estabilidade e solubilidade. A palavra “quelatos” vem do grego “chele” que significa “garra”, um termo adequado para descrever a maneira na qual os íons metálicos polivalentes são ligados a compostos orgânicos ou sintéticos (Mellor, 1964). Por sua vez, Ensminger & Oldfield (1990) definem quelatos como sendo estruturas cíclicas nas quais um elemento mineral é ligado a agentes carreadores ou quelantes através de ligações covalentes. Esses quelantes têm o papel de aumentar a absorção e a disponibilidade desse mineral no organismo, além de aumentar a sua estabilidade física reduzindo assim a tendência do micromineral de separar-se do alimento. Segundo Leeson & Summers (2001), existem três grupos de quelatos que são reconhecidos pelo sistema biológico:

Grupo I: Quelatos que servem de transportadores e de estoque para íons metálicos. Com este tipo de quelato o metal requer um ligante com propriedades químicas e físicas que o quelato é capaz de ser absorvido, transportado no sangue e passar pela membrana celular, enquanto o íon metal é utilizado no local em que é exigido. Ex: aminoácidos, especialmente a cisteína e a histidina e o EDTA.

Grupo II: Quelatos que são essenciais no metabolismo. Existe um número de quelatos no organismo com estrutura na qual o íon metal está presente na forma quelatada, a qual é necessária para desempenhar funções metabólicas. A hemoglobina é um exemplo deste grupo de quelatos.

Grupo III: Quelatos que interferem na utilização de cátions essenciais e não possuem valor biológico. Dentre estes quelatos está o ácido fítico (quelato de Zn) que pode interferir com o metabolismo normal por tornar esse mineral essencial indisponível para as funções metabólicas.

A "Association of American Feed Control Officials" – AAFCO (2000) define esses produtos minerais quelatados conforme abaixo:

- Quelato metal-aminoácido: é um produto resultante da reação de um sal metálico solúvel com aminoácidos na proporção molar, isto é, um mol do metal para um a três moles (preferencialmente dois) de aminoácidos na forma de ligação covalente coordenada. O peso molecular médio dos aminoácidos hidrolisados pode ser, aproximadamente, de 150 dáltons e o peso molecular resultante do quelato não deve exceder a 800 dáltons;
- Complexo aminoácido-metal: produto resultante da complexação de um sal metálico solúvel com aminoácido(s);
- Metal (Complexo aminoácido específico-metal): produto resultante da complexação de um sal metálico solúvel com um aminoácido específico;
- Metal proteinado: produto resultante da quelação de um sal solúvel com uma proteína parcialmente hidrolisada;
- Complexo metal-polissacarídeo: produto resultante da complexação de um sal solúvel com polissacarídeo.

Aparentemente, a grande vantagem dos minerais orgânicos é que a ligação do mineral a um ligante orgânico proporciona estabilidade do quelato no sistema gastrointestinal superior (Leeson & Summers, 2001). Comercialmente as formas orgânicas de diversos minerais como Zn, Mn e Cu são utilizados na nutrição animal, e a sua maior bioequivalência em comparação as fontes inorgâncias têm sido demonstrada (Cao et al., 2002; Paik et al., 1999; Wedekind, et al., 1992). Neste contexto, é importante ressaltar que os níveis de outros minerais dietéticos podem afetar a biodisponibilidade relativa (Scheideler, 1991), e que, diferentes ligantes orgânicos comercializados não irão necessariamente aumentar a biodisponibilidade de um dado mineral, é importante avaliar o ligante, origem, e estabilidade do mesmo (Cao et al., 2002).

Segundo Reddy (1992), as formas orgânicas aumentam a biodisponibilidade dos minerais em relação às formas inorgânicas, o que pode trazer benefícios, tais como: maior taxa de crescimento, maior ganho de peso, maior produção de ovos, melhora na qualidade de carne e ovos, redução da taxa de mortalidade e redução do efeito do estresse. Avaliando a progênie de reprodutoras pesadas suplementadas com fontes inorgânicas e orgânicas de Zn, há pesquisas onde não foram encontrados efeitos no desempenho produtivo da reprodutora (Hudson et al., 2004a; Kidd et al., 1992; Rebel et al., 2004; Virden et al., 2003). Entretanto, benefícios foram encontrados na progênie como: viabilidade (Virden et al., 2003), peso de tíbia (Kidd et al., 1992), e vários parametros imunológicos incluindo peso de timo (Kidd et al., 1992), reposta imune celular (Virden et al., 2003), ou resposta imune humoral (Kidd et al., 1993). Nestes trabalhos com Zn orgânico, o parâmetro de imunidade celular que apresentou um resultado consistente foi o de hipersensibilidade cutânea basófila (CBH), induzida por fito-hemaglutinina (Kidd et al., 1994, 2000), entretanto, outros trabalhos não demonstraram o mesmo efeito (Hudson et al., 2004b).

Nutrição maternal e sua influência no desenvolvimento embrionário

O estato mineral da galinha, é afetado pelo estato mineral da dieta, que por sua vez, esta correlacionado com a transferência de minerais para o ovo e utilização pelos embriões (Richards e Steele, 1987). Os minerais estão envolvidos em uma série de funções metabólicas e enzimáticas essenciais para a vida. Na literatura, existem vários trabalhos mostrando que a deficiência nutricional de uma série de minerais pode ocasionar deformações na formação do embrião, assim como, uma elevada mortalidade embrionária (Kidd, 2003). Entretanto, os sintomas clássicos da deficiência de microminerais observados no início da década de 20, não são muito usuais de serem encontrados hoje em dia, uma vez que, as empresas utilizam pré-misturas de microminerais com todos os elementos essencias para adequado crescimento.

Segundo Kidd (2003), pequisas foram conduzidas na aréa de macrominerais, como Ca e P, para otimizar o crescimento e aspectos reprodutivos relacionados à produção de reprodutoras. O balanço de eletrólitos como Na, potássio (K) e cloro (Cl), também foram estudados e são monitorados constantemente, a fim de evitar redução no desempenho reprodutivo das aves. Entretanto, é pouco provável que a faixa de variação de níveis nutricionais dos macrominerais mencionados acima impactem de forma significativa no crescimento e desenvolvimento da progênie. Pesquisas relacionadas na transferência de minerais da reprodutora para os ovos férteis, e posterior melhora do desenvolvimento e imunidade dos embriões, foram conduzidos primeiramente com microminerais (Richards et al., 1991; Kidd, 1992; Virden et al., 1997; Hudson et al., 2004ab;).

Existem na literatura revisões bibliográficas que discutem o metabolismo mineral em embriões de aves, as reservas de minerais no ovo, a localização desses armazenamentos, as quantidades de minerias presentes, como eles são mobilizados e utilizados pelo embrião (Richards, 1997; Kidd, 2003; Dibner et al., 2007). Entretanto, há poucas pesquisas publicadas relacionadas sobre o impacto da nutrição materna e o desenvolvimento esquelético da progênie (Kidd et al., 1992).

A maioria dos minerais estão localizados na gema do ovo e sua concentração não é fortemente influenciada pela concentração de minerais da dieta materna (Harms et al, 1964; Latchaw, 1975; Naber, 1979; Triyuwanta et al., 1992; Angel, 2007). Os últimos trabalhos de investigação do impacto da nutrição materna na concentração de microminerais no ovo abordaram a forma química do mineral e sua facilidade de utilização e mobilização pelo embrião ao invés de níveis nutricionais ótimos (Richards e Packard, 1996; Kidd, 2003; Hudson et al., 2004b; Dibner et al., 2007). Neste contexto, é importante ressaltar que, o embrião tem recursos minerais finitos para o desenvolvimento esquelético e que esses elementos são também necessários para o crescimento e desenvolvimento de outras funções, como o sistema imunológico por exemplo. Práticas de manejo nutricional, para modificar as quantidades totais de minerais depositados no ovo, podem estar relacionadas com as alterações no tamanho relativo da gema, no entanto, na prática, a modificação da forma química do mineral na dieta materna, sua transferência para o ovo, e um melhor acesso para a utilização do embrião parece ser uma ferramenta com um impacto positivo potencialmente maior. Avaliando o efeito do enriquecimento de minerais de ovos incubados utilizando o método de alimentação "*in ovo*" Yair & Uni (2011), obsevaram que ovos eriquecidos com fontes orgânicas de Fe, Zn, Mn, Cu, Ca e P inorgânico aos 17 dias de incubação exibiram uma incorporação significativa de Fe, Zn e Mn entre o 20° dia de incubação e o nascimento.

A casca também é uma importante reserva mineral para o embrião, entretanto, a maior parte do conteúdo da casca não é utilizado. A liberação de Ca e magnésio (Mg) da casca para o embrião já foi documentada (Packard & Packard, 1991; Richards, 1997). Mais recentemente, avaliando o conteúdo de mineral na casca no dia da incubação e no dia do nascimento, foi observado que as reservas de P, Fe, S, Na e Mn na casca dimuíram significativamente ao final dos 21 dias de incubação (Yair & Uni, 2011). No caso do Ca e o Mg, a casca fornece grandes quantidades ao embrião. Estima-se que cerca de 70 a 90% de Ca e 20 a 30 % do Mg utilizado pelo embrião vem da casca (Packard & Packard, 1991; Richards & Packard, 1996). Já reservas de P na casca são bastante pequenas e as quantidades disponíveis para todas as funções, incluindo a mineralização óssea do embrião, são encontrados principalmente na gema. Ao nascimento, uma quantidade significativa de P é retido no saco vitelino residual, o qual em parte tem uma grande importância na síntese de fosfolipídeos. Assim, a maior parte do P residual não esta disponível para o crescimento e mineralização óssea (Yair & Uni, 2011). A medida que o processo de incubação evolui, as concentrações de P e Mg diminuem na gema, enquanto que as concentrações de Ca aumentam a medida que o Ca é mobilizado da casca. (Romanoff, 1967; Richards & Packard, 1996). Como menores constituintes dos ossos, o Zn, Mn e Cu não são geralmente considerados na avaliação da mineralização óssea de embriões na produção comercial de reprodutoras pesadas, entretanto, seu potencial impacto para adequado crescimento ósseo já foi demonstrado (O'Dell et al., 1961; Kienholz et al., 1964; Leach, 1976). Além da essencialidade, pesquisas com estes minerais têm mostrado uma correlação direta entre o estato mineral da galinha e as respostas da progênie (Kidd, 2003; Hudson et al., 2004ab; Dibner et al., 2007).

Zinco

Como componente da anidrase carbônica, o Zn desempenha funções regulatórias no desenvolvimento ósseo (Kienholz et al., 1964; Kidd et al., 1992). O Zn é um dos microminerais essenciais para o crescimento embrionário de todas as espécies, inclusive aves. Esta envolvido na regulação da transcrição do DNA (Luscombe et al., 2000), a qual controla a diferenciação dos mais diferentes tipos de células. Outra função muito importante do Zn e fundamental para o desenvolvimento embrionário e pós-eclosão é a regulação do "turnover" celular. Em adição a estas funções, o Zn é constituinte ou ativador de centenas de enzimas sendo o mineral mais abundante nas metaloenzimas, além disso, é requerido para a síntese de duas proteínas funcionais chaves: colágeno e queratina (Underwood and Suttle, 2001). Estas duas proteínas são de grande importância para o desenvolvimento das aves, a queratina é a proteína estrutural da pele, penas, bico e unhas, enquanto que, o colágeno é a principal proteína estrutural dos tecidos de sustentação, incluindo cartilagem e ossos. Alguns dos principais e mais comuns sintomas da deficiência de Zn, estão relacionadas com estas duas proteínas estruturais como: mal formação de ossos e bico durante o período de desenvolvimento embrionário até o crescimento retardado, perose, descamação da pele e empenamento bastante empobrecido no período pós eclosão. Além disso, deficiência ou excesso de Zn estão ambas associadas com anorexia (Leeson e Summers, 2001).

Manganês

Essencial para a formação da cartilagem óssea, o Mn está envolvido na formação de proteoglicanos (Caskey et al., 1939), os quais na epífise óssea melhoram a resistência à compressão (Leach, 1976). Apesar do Mn ser amplamente distribuído no organismo, ele é encontrado em baixas concentrações nas células e tecidos, entretanto, extremamente necessário para o adequado desenvolvimento dos ossos e para a manutenção do processo reprodutivo de machos e fêmeas (Underwood & Shuttle, 2001).

O Mn, é ativador metálico das enzimas envolvidas na síntese de mucopolissacarídeos e glicoproteínas que contribuem na formação da matriz orgânica dos ossos e da casca dos ovos (Georgievski et al., 1982). Leach (1976), observou uma diminuição na produção de ovos e defeitos na formação da casca, quando galinhas foram submetidas a uma dieta deficiente em Mn. Os ovos apresentaram um menor peso de casca, com um formato mais circular e áreas translucentes. Neste mesmo sentido, ao retirar microminerais da dieta de poedeiras comercias durante 10 dias, Abdallah et al. (1994), observaram que o Mn parece ser o micromineral mais crítico quanto a qualidade de casca dos ovos, apresentando os mesmos problemas como formatos e áreas translúcidas da casca. A deficiência de Mn em frangos, perus e patos também pode ser manifestada pela incidência de perose (Gallup & Norris, 1939). A perose é caracterizada pelo alargamento e malformação da articulação tíbio-metatarseal, torção e flexão da tíbia, espessamento e encurtamento dos ossos longos.

É bastante questionável o quanto do Mn presente nos alimentos é disponível para as aves, a sua eficiência de absorção é bastante baixa. Outros minerais como Ca, P, e Fe podem reduzir a solubilidade do Mn no trato gastrintestinal. Além do que, o Mn compete pelos mesmos sítios de absorção do Fe e Cobalto (Co). A absorção e excreção de Mn parecem ser dependentes da formação de um quelato natural, especialmente com sais biliares (Leeson & Summers, 2001).

Cobre

Importante no desenvolvimento ósseo, o Cu tem uma importante função na formação da elastina e colágeno proporcionando uma maior elasticidade e resistência a tração da matriz óssea (O’Dell et al., 1961; Dibner et al., 2007). O Cu é essencial para a reprodução e o desenvolvimento embrionário das aves. Reprodutoras com deficiência severa de Cu na dieta apresentam defeitos na formação da casca do ovo (Baumgartner et al., 1978) e significativa redução na produção e eclodibilidade de ovos férteis (Savage, 1968). Embriões oriundos de reprodutoras que receberam dieta deficiente em Cu, apresentaram hemorragias, anemia, anormalidades no desenvolvimento ósseo e crescimento retartado. O efeito sobre a anemia parece ser devido a um efeito do Cu na mobilização e transporte de Fe (Leeson and Summers, 2001). Ao contrário do que acontece com o Zn, muitos efeitos da deficiência de Cu pode ser rastreado pelo seu papel com componente de várias metaloenzimas, ao invés de regulação da expressão gênica ou renovação celular. Por exemplo, o Cu afeta vários sistemas biológicos em virtude do seu papel importante para adequada produção de colágeno e elastina. A enzima lisil oxidase, é uma metaloenzima que é reduzida em animais que receberam dietas deficientes em Cu. O resultado é que a elastina e colágeno, de tais animais, não serão capazes de resistir as tensões mecânicas típicas dos sistemas cardiovascular e esquelético, onde os ossos podem ficar frágeis e serem facilmente quebrados (Guenthner et al., 1978; O’Dell el al., 1961).

Limitações nutricionais das aves durante o desenvolvimento embrionário

A diminuição da idade de abate de frangos, para atingir o peso de abate, tem aumentado a importância do período de incubação, a qual representa 33% em um frango abatido com 42 dias. Apesar do amplo conhecimento técnico existente a respeito de nutrição de frangos durante o período de crescimento, a informação existente sobre a nutrição do embrião durante o período de 21 dias de incubação é bem menor. O conhecimento sobre o estado nutricional do embrião durante a incubação, é essencial para a compreensão dos eventos que influenciam o desenvolvimento embrionário, eclodibilidade, qualidade dos pintinhos e o desempenho zootécnico da progênie (Uni et al., 2012).

Diferente dos mamíferos, o crescimento e desenvolvimento embrionário das aves, assim como, a eclosão são dependentes dos nutrientes depositados no ovo fertilizado. Embora o ovo fértil tem uma composição pré definida, as taxas e mecanismos de absorção desses nutrientes pelo embrião são desconhecidas. Além disso, os perfis de desenvolvimento e capacidades dos mecanismos fisiológicos, celulares e moleculares que digerem e absorvem os nutrientes não foram totalmente investigados (Richards, 1997).

O embrião das aves, deriva todas as suas necessidades de nutrientes durante o período de incubação do albumen, gema e casca. O albumen representa aproximadamente 65 a 75% do conteúdo total do ovo, consiste de aproximadamente 88% de água e 12 % de proteínas, as quais são totalmente consumidas pelo embrião durante a incubação (Romanoff, A. L. 1960; Shenstone, F. S. 1968). A gema consiste de aproximadamente 50% de água, 15% de proteínas, 33% de gordura e menos de 1% de carboidratos, entretanto, esta composição é dependete da idade da matriz, peso do ovo e genética da reprodutora (O'Sullivan et al., 1991; Vieira and Moran Jr., 1998).

Embora a absorção de lipídios pelo embrião, através do saco vitelino, tenha sido alvo de muitos estudos, pouco se sabe a respeito da absorção de proteínas, aminoácidos, carboidratos e minerais. A importância dos minerais, para o crescimento e desenvolvimento embrionário e progênie pós eclosão é suportado por várias publicações (Richards, 1997; Kidd, 2003; Dibner et al., 2007; Uni et al., 2012). Deficiências de um micromineral específico, pode ser facilmente induzido no desenvolvimento embrionário das aves alimentando as reprodutoras com quantidades insuficientes deste micromineral, que por sua vez, irá ocasionar a produção de ovos com níveis marginais deste elemento (Richards e Steele, 1987).

A exigência de minerais para reprodutoras pesadas para ótima produção de ovos, eclodibilidade, e qualidade de pintinhos não é definida pelo NRC (1994). Diversos trabalhos têm mostrado que severas deficiências de microminerais como Zn, Mn e Cu impactam em queda de produção de ovos, eclodibilidade e desordens de ordem esquelética, imunológica e cardiovascular, além de uma maior mortalidade durante o período embrionário e primeiros dias pós eclosão (Caskey et al., 1939; Kienholz et al., 1961; O'Dell et al., 1961). Entretanto, tem sido mostrado que alimentar as reprodutoras com elevadas concentrações de minerais tem pouco ou nenhum efeito sobre a concentração destes minerais no ovo (Naber, 1979; Angel, 2007), sendo que, alguns podem ser até tóxicos e causar efeitos deletérios, tanto na produção de ovos como no desenvolvimento embrionário.

Minerais no ovo

Os níveis de microminerais no ovo podem variar, e a quantidade total depositada no ovo é dependente da forma química do mineral e da quantidade fornecida para a galinha (Naber, 1979; Stadelman & Pratt, 1989). Geralmente, a maior porção de microminerais é depositada na gema, entretanto, pequenas quantidades também são depositadas na porção do albúmen do ovo (Richards, 1997). Alguns microminerais exibem uma distribuição variável entre a gema e o albúmen do ovo, dependendo da forma química do elemento disponibilizado para a galinha. Formas orgâncias e naturais de Se, por exemplo, como Se-metionina e levedura de Se, promovem uma maior deposição deste mineral no albúmen do ovo comparado a gema (Latshaw, 1975; Latshaw & Biggert, 1981; Swanson, 1987). De forma contrária, formas inorgânicas como o selenito resulta em uma maior quantidade de Se na gema comparado ao albúmen.

Parece estar elucidado na literatura que, dietas contendo níveis bastante elevados de microminerais com o Zn, Cu e Fe na forma inorgânica não aumentam de forma significativa a quantidade depositada no ovo (Elvehjem et al., 1929; Kienholz et al., 1964; Stahl et al., 1988). O fato é que, níveis muito elevados de Zn (Shippee et al., 1979; McCormick & Cunningham, 1984; Decuypere et al., 1988) e Cu (Stevenson et al., 1983), em dietas para poedeiras pode causar uma rápida paralização na produção de ovos, provocado por uma intoxicação alimentar destes minerais.

Ambos Zn e Mn têm uma distribuição similar no ovo, sendo que, sua deposição ocorre predominantemente na fração granular da gema (Richards, 1997). Comparado a estes dois minerais, uma alta percentagem de Cu é econtrada no albúmen, na casca e membranas interna do ovo. Contudo, a transferência de microminerais da galinha para o ovo envolve duas possíveis rotas: 1) via ovário para a gema, e 2) via oviduto para o albúmen, membrana da casca e casca (Richards, 1997). Devido a gema constituir a maior reserva de microminerais do ovo, a tranferência pelo ovário é claramente a principal rota para o armazenamento destes nutrientes essenciais (Uni et al., 2012).

A circulação dos microminerais para o ovário, e consequentemente para a gema do ovo, envolve a produção de vitelogenina, um estrógeno que é induzido por uma proteína precursora da gema, a qual é produzida pelo fígado das galinhas e secretada na corrente sanguínea. Foi observado que, a medida que aumentam os níveis plasmáticos de vitelogenina, aumentam os níveis de uma série de microminerais (Hill, 1974; Richards, 1989). Esse aumento na circulação de microminerais podem ser contabilizada através da ligação entre vitelogenina e o metal (Richards & Packard, 1996). Além disso, tem sido sugerido que os níveis plasmáticos de Zn podem ser utilizados como um indicador indireto dos níveis de vitelogenina presentes no plasma de poedeiras (Mitchell & Carlisle, 1991).

Embora a fração granulosa da gema seja responsável pelo maior percentual de microminerais na gema, existe também alguma acumulação de minerais na fração solúvel da gema também chamada de livetina (Burley & Vadehra, 1989). Esta fração da gema, em particular, é originada a partir de proteínas plasmáticas derivadas do plasma da galinha e constituida por tês subfrações denominadas a, b e g correspondentes a albumina plasmática, glicoproteínas e imunoglobulinas, respectivamente (Williams, 1962). O papel da transferrina de origem materna, em fornecer uma fonte de Fe e talvez outros minerais para o embrião em desenvolvimento, ainda não foi estabelecida. Entretanto, utilizando uma transferrina marcada Frelinger (1971), demonstrou que a maior parte da transferrina em circulação de um pombo recém eclodido era derivado do plasma materno transferido através do ovo.

Uma segunda rota potencial de transferência de microminerais para os ovos, envolvem o oviduto e sua síntese de albumina, membrana da casca e a casca propriamente dita. O estrogênio em conjunto com outros esteróides gonodais (progesterona e testosterona) estão envolvidos na indução da síntese de proteína da albumina através das células glandulares tubulares da porção do magno do oviduto (Palmiter, 1972). A casca e suas membranas associadas contém uma quantidade variável de Zn, Mn, Cu e Fe (Mas & Arola, 1985; Richards, 1991). A membrana da casca de galinhas e perus contém uma alta concentração de Cu (Baumgartner et al., 1978; Richards, 1991). Além disso, poedeiras alimentadas com uma dieta deficiente em Cu resultaram em uma formação estrutural anormal da membrana da casca, além de um redução significativa de sua concentração (Baumgartner et al., 1978).

Por outro lado, foi demonstrado *in vitro* que a membrana da casca do ovo da galinha, apresenta uma elevada afinidade de ligação para uma variedade de metais pesados (Suyama et al., 1994) e talvez essa seja uma rota de excreção dos mesmos pela galinha. Embora a casca do ovo fornece uma quantidade significativa de Ca para o embrião durante o seu desenvolvimento, a importância, se houver, de depósito de microminerais na casca do ovo para a nutrição embrionária não esta elucidada. Se estes depósitos são ativamente mobilizados como fontes distintas ou passivamente como parte da mobilizaçao do Ca, também é um assunto desconhecido. Embora tenha sido sugerido que o depósito de metais pesados na casca representa uma rota de excreção da galinha (Burger, 1994), a importância dos microminerais essenciais depositados na casca do ovo e suas membranas, não deve ser descartada sob o ponto de vista de nutrição do embrião.

Membranas extra-embrionárias

Devido as membranas extra-embionárias estarem em contato direto com a gema, albúmen e casca, as membranas extra-embrionárias desempenham um papel crucial na mobilização e transferência de minerais dos locais de armazenamento do ovo para os tecidos do embrião em desenvolvimento (Richards et al., 1991). Nenhuma função foi atribuída à membrana amniótica no que diz respeito ao metabolismo de minerais, apesar de que, o líquido amniótico é rico em minerais como o Fe, por exemplo (Romanoff, 1967). Por sua vez, a membrana do saco vitelino tem um papel bastante importante na nutrição mineral do embrião das aves (Romanoff, 1967).

A mobilização de microminerais da gema envolve, em grande parte, a captação e processamento de grânulos de vitelo pela endoderme do revestimento do saco vitelino epitelial. Vários estudo utilizando microscopia eletrônica, demonstraram que, os grânulos de gema são levados para dentro da célula por endocitose endodérmica (Lambson, 1970; Mobbs & McMillian, 1979). Outra função bem documentada do saco vitelino é a sua capacidade de sintetizar e secretar proteínas plasmáticas (Kram e Klein, 1976), incluindo os que estão envolvidos no transporte de minerais, tais como a transferrina, albumina e alfa-fetoproteína (Young e Klein, 1983). Por isso, propõe-se que a membrana do saco vitelino não é simplesmente uma barreira passiva entre o embrião e gema, mas sim, que tem a habilidade de absorver os microminerais da gema, armazená-los durante períodos curtos de tempo, e subsequentemente os liberar para os tecidos embrionários através da circulação vitelina (Richards, 1997).

Trabalhos mostram que, a concentração sérica de microminerais como Zn, Mn e Cu diminuem significativamente tanto na gema como no saco vitelino durante o desenvolvimento embrionário de frangos e perus, indicando uma mobilização ativa de microminerais contidos nestes locais de reserva (Richards, 1997; Yair & Uni, 2011). Estas alterações podem refletir bem as flutuações nos níveis de transferina, albumina e alfa-fetoproteína que são conhecidas por ocorrer no plasma de embriões de aves durante a última metade do desenvolvimento (Kimura, 1983, Richards, 1991).

Sendo assim, a coordenação da mobilização de microminerais entre a gema e o saco vitelino são de extrema importância para regular o fornecimento deste minerais para o embrião, de acordo com a exigência de cada tecido específico e também pela taxa de crescimento e fase do desenvolvimento embrionário.

Tecidos embrionários

Existem uma série de trabalhos, que mostram a transferência de microminerais do ovo para o embrião de pintos em desenvolvimento (Gallup & Norris, 1939; McFarlane & Milne 1934; Ramsay, 1951). Sabe-se que, a medida que o embrião vai se desenvolvendo a concentração de minerais no ovo diminuem (Dewar et al., 1974). As concentrações elevadas dos minerais no ínicio da incubação, provavelmente refletem diferentes mecanismos de transferência, dependendo do estágio de desenvolvimento do embrião. Entre os dias 5 e 10 de incubação, por exemplo, o saco vitelino ainda está em desenvolvimento para o seu pleno funcionamento no terço final da incubação, logo, a transferência de minerais para o embrião durante este período talvez não seja regulada por este segmento. Além disso, é também possível que nos estágios iniciais do desenvolvimento embrionário das aves, seja necessário uma alta concentração de microminerais disponíveis para suportar uma rápido crescimento durante este período (Romanoff, 1967).

Durante o desenvolvimento embrionário das aves, o fígado é o órgão de maior importância no armazenamento e regulação homeostática do metabolismo mineral (Richards & Steele, 1987). As concentrações hepáticas de Zn e Fe em embriões de perus diminuiram significativamente entre os dias 14 e 28 de incubação, enquanto que, os níveis de Cu no fígado chegaram ao seu pico aos 23 dias de incubação antes de diminuir até o período do nascimento (Richards & Weinland, 1985). Alterações semelhantes, foram observadas para os níveis de Zn e Cu no fígado de embriões de galinhas (Sandrock et al., 1983; Fleet & McCormick, 1988). Logo, as funções hepáticas possuem uma grande importância na manutenção e homeostase dos minerais durante o desenvolvimento embrionário, talvez, servindo como um local de armazenamento rápido, de curto prazo, para quantidades em excesso derivadas da gema, antes de sua transferência para os tecidos alvo do embrião (Richards, 1997). Entretanto, não há estudos que tenham seguido especificamente a rota de distribuição hepática para os tecidos de cada micromineral via complexação no plasma com proteínas transportadoras.

Desenvolvimento esquelético do embrião

A mineralização do esqueleto em embriões de frangos de corte inicia-se ao oitavo dia de incubação, sendo a gema a fonte primária de Ca neste período (Richards & Steele 1987). Na produção de frangos de corte, os nutrientes transferidos pela reprodutora ao ovo durante a sua formação é de fundamental importância para o desenvolvimento do embrião. O conhecimento de como a alimentação das reprodutoras influencia o desenvolvimento fisiológico do embrião, pode justificar a inclusão de determinados ingredientes na formulação de ração com o intuito de melhorar a qualidade de pintinhos de um dia de idade.

Alterações nos membros locomotores de frangos de corte podem estar associadas a desordens metabólicas iniciadas durante o período embrionário (Dibner et al., 2007). O desenvolvimento esquelético, denominado ossificação endocondral, é o processo de formação dos ossos a partir de cartilagem pré-existente (Patten & Carlson, 1974). O processo de conversão da cartilagem hialina em esqueleto desenvolve-se durante o período embrionário e finaliza-se após a eclosão. Desta forma, a ossificação endocondral é formada a partir de dois processos: modificação da cartilagem hialina, e posterior invasão de células mesenquimatosas que se diferenciam em osteoblastos (Patten & Carlson, 1974; Dibner et al., 2007). Durante o processo de ossificação endocondral, o tecido cartilaginoso é precursor do tecido ósseo. Este tecido sofre vários graus de mineralização, proporcionando rigidez não observada em outros tecidos. O esqueleto da forma, estrutura e especialmente suporte para os músculos, sendo que, o crescimento muscular está diretamente relacionado ao tamanho do esqueleto (Leeson & Summers, 2001).

Por esta razão, o objetivo deste projeto de tese foi avaliar os efeitos da suplementação dos microminerais orgânicos Zn, Mn, Cu e sua associação à constituição dos ovos e tecidos de sustentação, ossos e cartilagens, dos embriões em diferentes fases de desenvolvimento.

Hipóteses e objetivos

Hipóteses

A produtividade de reprodutoras pesadas aumenta com a inclusão dos microminerais na forma orgânica.

A suplementação de Zn, Mn e Cu na forma orgânica aumenta as concentrações desses minerais no albúmen e gema dos ovos em comparação a fontes inorgânicas.

A eclodibilidade e a qualidade de pintinhos aumentam com a utilização de minerais na forma orgânica em dietas fornecidas à sua geração parental. O desenvolvimento esquelético embrionário e pós-eclosão melhores nas aves oriundas de matrizes que recebem minerais orgânicos na dieta.

Objetivos

Avaliar os efeitos da suplementação de Zn, Mn e Cu complexados a aminoácidos ou não em dietas de reprodutoras pesadas sobre:

- Produção de ovos, ovos incubáveis, qualidade e espessura de casca;
- Composição mineral da clara e albúmen dos ovos e verificar se há correlação com os tratamentos em estudo;
- Eclodibilidade, mortalidade embrionária e qualidade dos pintinhos gerados, bem como, o desenvolvimento esquelético dos embriões em diferentes períodos da incubação e ao nascimento.

CAPÍTULO II[1]

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Reproductive performance of Cobb 500 breeder hens fed diets supplemented with zinc, manganese and copper from inorganic and amino acid-complexed sources

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Primary Audience: Nutritionists, Broiler Breeder and Hatchery Managers, Researchers

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SUMMARY

The objective of this study was to investigate the effects of maternal dietary Zn, Mn and Cu source on egg production, egg shell quality, hatchability, and hatched chick grading. Inorganic sources of Zn, Mn, and Cu (IZMC) as zinc sulfate monohydrate (35% Zn), manganese sulfate monohydrate (31% Mn), and copper sulfate pentahydrate (25% Zn) or organic sources of Zn, Mn, and Cu as amino acid-mineral complex (OZMC) were used. The 3 experimental treatments consisted of diets supplemented with: 1) 100, 100 and 10 mg/kg of Zn, Mn, and Cu, respectively from IZMC (CONTROL); 2) 60, 60, and 3 mg/kg of Zn, Mn, and Cu, respectively from IZMC plus 40, 40, and 7 mg/kg of Zn, Mn, and Cu, respectively from OZMC (ISO); and 3) a diet with 100, 100 and 10 mg/kg of Zn, Mn, and Cu, respectively from IZMC as in CONTROL plus 40, 40, and 7 ppm supplemental Zn, Mn, and Cu from OZMC (ON TOP). Treatments were fed from 22 to 68 wk of age. Each treatment had 10 replications of 20 females and 2 males. Feeding the ISO compared to the CONTROL diet increased egg shell weight and thickness ($P < 0.05$), and decreased early embryo mortality ($P < 0.01$). Feeding the ON TOP treatment compared to the CONTROL diet resulted in thicker and heavier egg shells ($P < 0.05$). There was an improvement in egg shell quality in breeder hens consuming OZMC supplemented ISO or ON TOP with IZMC.

DESCRIPTION OF PROBLEM

Trace minerals such as Zn, Mn, and Cu are components of eggs that are essential for broiler breeder performance as well as chicken embryo development [1, 2]. These trace minerals are constituents of several proteins involved in intermediary metabolism, hormone secretion, the immune system [3], and are required in small amounts in the diet. The effects of Zn, Mn, and Cu deficiencies on breeder performance and embryo development have been well documented and can result in low egg production, reduced egg shell strength, poor hatchability, reduced fertility, increased embryo bone abnormalities, poor feathering and dermatitis [4-6]. Mineral concentration in conventional feedstuffs can fluctuate widely due to the trace mineral composition in soils, geographic area, climate, crop [7]. Thus, in poultry practical formulations, nutritionists usually include a wide margin of safety for trace minerals to ensure appropriate animal growth and maximize performance [8].

In breeder hens, Zn is important as a component of carbonic anhydrase, which is involved in the supply of carbonate ions during egg shell formation [9]. Other important Zn metalloenzymes include carboxypeptidases and DNA polymerases, which are important in immune responses, skin and wound healing, and hormone production. Breeder diets that are deficient in Zn can lead a decrease in egg production and egg shell quality, hatchability [4]. Essential for formation of the bone cartilage, Mn plays a significant role in the formation of chondroitin sulfate. Mn-deficient avian embryos exhibit shortening of the long bones, parrot beak, and wiry down [5]. The involvement of Cu in the synthesis of hemoglobin, erythrocuprein, and other plasma proteins is well known [10]. Moreover, Cu is closely associated with iron metabolism as it is a part of ceruloplasmin. Also Cu plays an important role in egg shell membrane formation, which in turn influences egg shell structure, texture and shape [11].

The trace minerals are traditionally supplemented in broiler feeds using inorganic sources (ITM), such as oxides or sulfates [12]; however, these sources have variable bioavailability [13, 14], which can impair the animal's performance and may contribute to environmental pollution. During transit through the gastrointestinal system, ions from dissolved ITM can potentially bind with other dietary components, forming insoluble complexes that are excreted thus reducing their availability [14, 15, 16]. The organic trace minerals (OTM) have been increasingly used in avian nutrition because they seem to have higher bioavailability compared to inorganic sources [13, 16]. These type of minerals have been shown to improve bird performance, enhance immunity and can potentially reduce mineral in excreta [17-21]. However, data from studies conducted with broiler breeders evaluating the effects of OTM are still limited.

The NRC [22] does not provide specific recommendations of Zn, Mn, and Cu for broiler breeder hens, instead fixed amounts are recommended for meat-type chickens until 8 wk, Leghorn-type laying hens, growing, holding and laying turkeys with the exception of Cu requirements for Leghorn-type laying hens. The NRC [22] recommendation do not take mineral availability into consideration, which can change the broiler breeder and progeny performance. Currently, the main source of information used by breeder nutritionists to set Zn, Mn, and Cu concentrations in breeder diets derive from primary broiler breeder manuals [23] since a few recent research reports exist in this area [17-20, 24]. The objective of this study was to evaluate the reproductive response of broiler breeder hens fed sulfate and amino acid-complexed sources of Zn, Mn, and Cu in breeder diets.

MATERIALS AND METHODS

Bird Husbandry

Birds in the study were managed according to the directives of the Ethics and Research Committee of the Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. Six hundred slow-feathering (SF), Cobb 500 broiler breeder hens and sixty Cobb 500 breeder males, 22 wk of age, were obtained from a commercial breeder farm [25]. Birds were individually weighed and placed in 30 floor pens in a tunnel-ventilated house, 20 females and 2 males per pen. Pens were 2.0 x 2.5 m and had 5 nipple drinkers, separate male and female feeders, and six nests located at one side of the pen. Forty-eight males were maintained in separated floor pens to replace sexually inactive or dead males. Litter was of new rice hulls 15 cm deep at placement. Animals were monitored daily and any abnormalities were recorded and treated as necessary.

Lighting and feeding throughout the study were provided according to primary breeder recommendations [26]. All birds were individually weighed every 4 wk for assessment of BW and uniformity. According to the BW and development, males and females were feed restricted following the recommended daily allowances for each sex. All dietary treatment groups were provided the same amount of feed on a per bird basis and water was provided for *ad libitum* consumption.

Experimental Diets

Two diet phases were used (22 to 32 wk and 33 to 68 wk) whereas males were fed the same diet throughout the entire study (Table 1). Inorganic sources of Zn, Mn, and Cu (IZMC) as zinc sulfate monohydrate (35% Zn), manganese sulfate monohydrate (31% Mn), and copper sulfate pentahydrate (25% Zn) or organic sources of Zn, Mn, and Cu as amino acid-mineral complex (OZMC) were used. The 3 experimental treatments consisted of diets supplemented with: 1) 100, 100 and 10 mg/kg of Zn, Mn, and Cu, respectively from IZMC (CONTROL); 2) 60, 60, and 3 mg/kg of Zn, Mn, and Cu, respectively from IZMC plus 40, 40, and 7 mg/kg of Zn, Mn, and Cu, respectively from OZMC (ISO); and 3) a diet with 100, 100 and 10 mg/kg of Zn, Mn, and Cu, respectively from IZMC as in CONTROL plus 40, 40, and 7 ppm supplemental Zn, Mn, and Cu from OZMC (ON TOP). A commercially available OZMC was used [27]. Each dietary treatment was replicated 10 times (3 dietary treatments in 30 floor pens). Formulated and analysed nutrient content of the diets are shown in Table 1.

The diets were manufactured every 4 wk. Diets samples were collected and analyzed, in duplicate, for CP, Ca, and P [28], Table 1. Diet subsamples and ingredients such as corn, soybean meal and wheat bran meal were analyzed, in duplicate, for Zn, Mn, and Cu concentration from each feed mix batch (4 wk). Drinking water samples were collected at the nipple drinker (breeder house) and analyzed, in duplicate, for Zn, Mn, and Cu. All trace minerals analysis were performed using inductive coupled plasma atomic emission spectroscopy (ICP-AES) [29] (Table 2).

Hen Performance Measurements

Eggs were collected 4 times daily, recorded as nest- or floor-laid and classified as settable, cracked, shell-less, double-yolk or abnormally shaped, regardless the location of lay (nest or floor). Mortality was recorded daily and feed allowance adjusted accordingly. Total and settable egg production on a hen-housed and hen-day basis and cumulative mortality (total number of dead birds / initial number of birds x 100%) were summarized weekly. Average egg weight and specific gravity were determined at 35, 45, 55 and 65 wk of age using all settable eggs nest-laid on a single day. Specific gravity was measured using saline solutions with concentrations ranging from 1.065 to 1.095 in intervals of 0.005 units [30]. Egg shell percentage and thickness were measured using 5 settable eggs from a single day, per replication, at 35, 45, 55, 65 wk of age. Egg shell weight was measured after washing, drying overnight at 105℃, with egg shell membrane on. Egg shell thickness was measured at three points at the eggs equatorial zone after inner membrane removal.

A number of 90 eggs per replicate pen were set for incubation every three wk, between breeder ages of 45 to 68 wk. Only nest laid-eggs that were not dirty, double-yolked, misshapen, broken, cracked, excessively small, or eggs with poor shell quality were incubated [31]. The eggs were stored during 7 d in a controlled environment room at 18 ℃ and 75% relative humidity, prior to incubation. The settable eggs were placed into replicate trays (n = 30 trays), which were randomly placed in a single-stage incubator [32] with 3,600-egg-capacity. The incubator was set as 37.5 ºC and 60% relative humidity. On day 18.5, eggs were transferred to the hatcher [32], which was set at 36.5 ºC and 65% relative humidity. Number of eggs that hatched was recorded at 21.5 d of incubation.

All incubated eggs that were cracked prior to incubation or at transference to the hatcher were removed from the data set. All unhatched eggs were broken open to determine the approximate day of embryonic death. Embryonic mortality was grouped into 4 categories: early (0 to 7 d), middle (7 to 14 d), late (15 to 21 d) and at piping. Hatchability of fertile eggs was expressed as number hatching chicks per fertile eggs set, and percentage cumulative hatchability was expressed as percentage hatching chicks to the total eggs set. Chicks per hen housed were estimated by multiplying weekly settable eggs per hen-housed per period and hatchability of total eggs set per batch set.

All hatched chicks were counted, feather-sexed and chick quality was visually assessed according to commercial hatchery standards [33]. This evaluation was conducted on all incubations and always by the same personnel and classified as saleable chicks, unhealed navels, culled (weak or red hocks), and physical abnormalities. Additionally, 200 newly hatched chicks (21.5 d incubation) per treatment (n = 10 each sex per replicate pen) were weighed (average) and individually measured from the tip of beak to end of middle toe (third toe) to determine the chick length as described by Molenaar et al. [34].

Statistical Analysis

Birds were randomly allocated to floor pens that had previously been randomly assigned to the different diet treatments. Each pen was a replicate. All reproductive performance data were taken on the same experimental units, repeated in time. All percentage data were subjected to angular transformation to stabilize variances (arcsine square root percentage transformation) before statistical analysis [35]. Repeated measurements analyses were applied to examine and compare response over time in a factorial experiment with diet treatment and time as the two factors.

Data were analyzed using the repeated statement PROC MIXED of SAS [36], adding the factor time as a fixed effect and pen was considered as a random effect [37]. The following statistical model was used:

$$Y_{ijk} = \mu + \alpha_i + d_{ij} + t_k + (\alpha t)_{ik} + e_{ijk}$$

where y_{ijk} is the response at time k on experimental unit j in treatment group i, μ is the overall mean, α_i is a fixed effect of treatment i, d_{ij} is a random effect of experimental unit j in treatment group i, t_k is a fixed effect of time k, $(\alpha t)_{ik}$ is a fixed interaction effect of treatment i with time k, and e_{ijk} is random error at time k on experimental unit j in treatment i. The best covariance structure was based on the Akaike Information Criteria (AIC) [38], and used the smallest AIC value for each variable [39]. The autoregressive covariance structure (AR[1]) fit the data best for hen-day egg production, hen-housed egg production, total eggs per hen-housed, settable eggs per hen-housed, egg weight, specific gravity, egg shell weight, egg shell thickness and hatchling chick weight and length. The heterogeneous autoregressive covariance structure (ARH[1]) fit the data best for fertility, hatchability of egg set, hatchability of fertile, chicks per hen-housed, saleable chicks, culls, unhealthy navels, and physical abnormalities. The unstructured (UN) fit the data best for embryo mortality measurements. Differences were determined using the PDIFF option of the LSMEANS statement and the Tukey-Kramer adjustment for multiple contrasts for all pairwise comparisons [36]. In all analyses, significance were declared at $P < 0.05$.

RESULTS AND DISCUSSION

Formulated and analyzed diet nutrients were similar (Table 1), except in the basal diet of the males, which was formulated to contain 13.77% of CP but analyzed as 14.21%. Increases in Zn, Mn, and Cu from the CONTROL and from ISO to ON TOP supplementation occured as expected. Analyzed Zn, Mn, and Cu composition of corn, soybean meal and wheat bran are presented in Table 2.

At the start of the experiment with 22 wk of age BW and uniformity were similar between replications (Figure 1). Mean BW and uniformity were not affected by treatments ($P > 0.05$; data not shown). Average livability from 24 to 68 wk was 5.3 % higher (98.2 vs 93.0%) as compared to the breeder strain guide [23], and was not affected by treatments ($P < 0.05$; data not shown). Breeder production (hen day egg production, hen housed egg production, eggs per hen housed and settable eggs per hen housed) was affected only by period ($P < 0.001$; Figure 2; Table 3). No period x diet interaction was observed for any of the parameters evaluated in this study ($P > 0.05$). All production data were affected similarly by period with an initial increase from period 1 to period 2, coinciding with peak production and a subsequent decrease between period 2 and 3 and a further decrease between period 3 and 4. As compared with the expected from the primary breeder manual peak of production was 4% higher (84.9 vs 81.5%) and the postpeak egg production was superior [23]. In addition, the number of eggs per hen-housed (from 25 to 68 wk) was 7.4% higher (192.9 vs 178.7) compared to the breeder strain from 24 to 65 wk [23]. When compared to commercial data, hen-day egg production could be considered typical for Brazilian broiler breeder flock.

All egg shell quality measures were affected by period ($P < 0.001$; Table 4). Relative egg shell weight decreased after 45 wk ($P < 0.001$), and the specific gravity and egg shell thickness decreased after 35 wk of age ($P < 0.001$). Egg shell relative weight and egg shell thickness were affected by diet ($P < 0.05$), however no differences were found in the egg specific gravity ($P > 0.05$). Eggs laid by hens feeding ISO or ON TOP treatments increased relative egg shell weight and thickness ($P < 0.05$) compared with the CONTROL diet. Specific gravity method is frequently used as an indicator of egg shell quality and considered to be adequate above 1.080 [8]. The relative egg shell weight and thickness is quite high in all treatments, which do not suggest that the CONTROL diet is negatively influencing shell formation in this study. Failure to show a consistent relationship between relative egg shell weight and thickness with the specific gravity may be due to large differences between test sensitivities.

The role of trace minerals in poultry nutrition and embryo development is well documented [1, 2, 40], however less is known about the specific involvement in the actual process of shell formation. Both Zn and Mn act as co-factors in the enzymes needed for calcium metabolism (i.e. carbonic anhydrase). The shell and its associated membranes have varying amounts of Zn, Mn, but contain an especially high concentration of Cu [1, 11]. Moreover, have been shown that Cu deficient diet can influence egg shell membrane structure, which in turn influences egg shell structure, texture, shape, pigments, and egg weight by an increase in the thin albumen portion [11]. It is important to consider that mineral deficiency does not appear to affect the absolute amount of shell deposited by the hen, but that variation in egg size impair in the shell thickness [41]. This appears to be a plausible explanation in the present study for the increase in egg weight and eggshell quality measurements as the flock aged from period 1 to period 4.

Egg mineral composition is variable and the total amount deposited in egg components may depend on the chemical form of the mineral and the amount fed to the hen [15, 42]. According to Richards [1] the transfer of trace minerals from the hen to the egg involves two possible routes: 1) via the ovary to the yolk, and 2) via the oviduct to the albumen, the egg shell and its membranes. Feeding hen diets with high levels of Zn, Mn and Cu does not mean that the egg mineral composition will increase significantly [4, 42]. Whereas, it has been shown that OTM sources of Zn, Cu, and Se as opposed to ITM respective sources result in higher total amounts of these minerals deposited into the egg [15, 20, 43, 44]. A significant improvement in the egg specific gravity and egg shell thickness was seen using a racemic mixture of Zn-sulfate and Zn-AA complex compared to a control diet containing only Zn-sulfate [20, 45]. Moreng et al., [46] found that feeding laying hens zinc methionine complex improved egg shell relative weight and thickness. Based on these results it can be postulated a synergistic effect when using a mixture of amino acid-complexed and inorganic sources of trace minerals and that this synergy may be due to enhanced mechanisms involved in calcification of egg shells [20].

Fertility, hatchability and chicks per hen-housed were affected only by period ($P < 0.001$; Table 5). Embryo mortality (early, middle, and late dead) was affected by period ($P < 0.05$; Table 5), however a reduction in the early embryo mortality was observed in eggs laid by hens fed the ISO treatment compared to the CONTROL diet ($P < 0.05$). Similarly, Hudson et al., [20] found a significant reduction in early embryo mortality when breeder hens were fed with ZnAA as the only source of Zn supplementation and also 3.6 more chicks per hen-housed in hens consuming diets supplemented with a racemic mixture of Zn-sulfate and ZnAA complex compared to the hens consuming only the ZnAA-supplemented diet. The Zn, Mn, and Cu play important roles in embryo development as well as hatchability [2, 4, 5, 40] and a positive relationship between egg Zn content and hatchability has been made [47]. Kidd et al. [17] found an increase in fertility in hens fed a diet supplemented with ZnAA when compared to a control diet (without Zn supplementation), however only a numerical response for hatchability was observed by them.

At hatch chick grades (saleable chicks, culls, and unhealthy navels), hatchling weight and length were affected only by period ($P < 0.001$), except for physical abnormalities where there was no period effect ($P > 0.05$; Table 6). No diet effect was observed in these parameters ($P > 0.05$). As expected, hatchling weight and length increased with flock age as a consequence of the increasing in the egg weight [48].

The improvement on relative egg shell weight, egg shell thickness, as well the reduction on early embryo mortality when broiler breeder hens were fed ISO or ON TOP treatment may be due to an increased the bioavailability of these minerals in the intestinal lumen. There is a hypothesis that OTM can resist dissociation in the relatively low pH of the proventriculus and gizzard, thus allowing the intact complex to be delivered to the absorptive epithelium of the small intestine [49]. This may result in decreases in antagonistic interactions between minerals and the binding with nutrients and non-nutritive components of the digesta [2]. Therefore, it is likely that Zn, Mn and Cu bound to certain stable ligands could improve the reproductive performance of breeder hens. However, more detailed information about trace minerals metabolism is needed to clarify the difference between the OTM, ITM source and their interaction when associated in breeder feed.

CONCLUSIONS AND APPLICATIONS

1. Broiler breeders provided the ISO treatment improved egg shell weight and thickness and reduced the early embryo mortality than the hens consuming the CONTROL diet.
2. Hens consuming the ON TOP supplementation resulted in thicker and heavier egg shells compared to the CONTROL diet.
3. Total or partial replacement of sulfates with organic trace minerals could potentially reduce manure trace minerals levels without any negative effects on various aspects of broiler breeder production, fertility, or hatchability.

REFERENCES AND NOTES

1. Richards, M. P. 1997. Trace mineral metabolism in the avian embryo. Poult. Sci. 76:152-164.

2. Dibner, J. J., J. D. Richards, M. L. Kitchell, and M. A. Quiroz. 2007. Metabolic challenges and early bone development. J. Appl. Poult. Res. 16:126-137.

3. Savage, J. 1968. Trace minerals and avian reproduction. Fed. Proc. 27:927-931.

4. Kienholz, E., D. Turk, M. Sunde, and W. Joekstra. 1961. Effects of zinc deficiency in the diets of hens. J. Nutr. 75:211-213.

5. Caskey, C., W. Gallup, and L. Norris. 1939. The need for manganese in the bone development of the chick. J. Nutr. 17:407-410.

6. Bird, D. W., B. L. O'Dell, and J. E. Savage. 1963. Copper deficiency in laying hens. Poult. Sci. 42:1256.

7. Hodgson, J. F., R. M. Leach, Jr., and W. H. Allaway. 1962. Micro-nutrients in soils and plants in relation to animal nutrition. Agr. Food Chem. 10:171-174.

8. Dale, N., and C. F. Strong Jr. 1998. Inability to demonstrate an effect of eggshell #49 on shell quality in older laying hens. J. Appl. Poult. Res. 7:219-224.

9. Nys, U., M. T. Hincke, J. L. Arias, J. M. Garcia-Ruiz, and S. E. Solomon. 1999. Avian egg shell mineralization. Poult. Avian Biol. Rev. 10:143-166.

10. Leeson, S. 2009. Copper metabolism and dietary needs. World´s Poult. Sci. J. 65:353-366.

11. Baumgartner, S., D. Brown, E. Salvsky, Jr., and R. Leach Jr. 1978. Copper deficiency in the laying hen. J. Nutr. 108:804-811.

12. Vieira, S. L., 2008. Chelated minerals for poultry. Rev. Bras. Cienc. Avic. 10:73-79.

13. Wedekind, K. J., A. E. Hortin and D. H. Baker. 1992. Methodology for assessing zinc bioavailability: Efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. J. Anim. Sci. 70:178-187.

14. Edwards, H. M., and D. H. Baker. 1999. Bioavailability of zinc in several sources of zinc oxide, zinc sulfate, and zinc metal. J. Anim. Sci. 77:2730-2735.

15. Dobrzanski, Z., K. Mariusz, C. Katarzyna, G. Henryk, and O. Sebastian. 2008. Influence of organic forms of cooper, manganese and iron on bioaccumulation of these metals and zinc in laying hens. J. Elementol. 13(3):309-319.

16. Cao, J., P. R. Henry, S. R. Davis, R. J. Cousins, R. D. Miles, R. C. Little, and C. B. Ammerman. 2002. Relative bioavailabity of organic zinc sources based on tissue zinc and metallothionein in chicks fed conventional dietary zinc concentrations. Anim. Feed Sci. Technol. 101:161-170.

17. Kidd, M. T., N. B. Anthony, Z. Johnson, and S. R. Lee. 1992. Effect of zinc methionine supplementation on the performance of mature broiler breeders. J. Appl. Poult. Res. 1:207-211.

18. Virden, W. S., J. B. Yeatman, S. J. Barber, C. D. Zumwalt, T. L. Ward, A. B. Johnson, and M. T. Kidd. 2003. Hen mineral nutrition impacts progeny livability. J. App. Poult. Res. 12:411-416.

19. Hudson, B. P., W. A. Dozier III, J. L. Wilson, B. D. Fairchild, and R. J. Buhr. 2004. Breeder age and zinc source in broiler breeder hen diets on progeny characteristics at hatching. J. Appl. Poult. Res. 13:55-64.

20. Hudson, B. P., W. A. Dozier III, J. L. Wilson, J. E. Sander, and T. L. Ward. 2004. Reproductive performance and immune status of caged broiler breeder hens provided diets supplemented with either inorganic or organic source of zinc from hatching to 65 wk of age. J. Appl. Poult. Res. 13:349-359.

21. Tako, E., P. Ferket, and Z. Uni. 2005. Changes in chicken intestinal zinc exporter mRNA expression and small intestinal functionality following intra-amniotic zinc-methionine administration. J. Nutr. Biochem. 16:339-346.

22. National Research Council. 1994. Nutrient requirements of poultry 9th revised edition. National Academy Press, Washington D.C.

23. Cobb-Vantress. 2008. Cobb 500 SF Breeder Management Supplement. Cobb Vantress Inc., Arkansas, USA.

24. Kidd, M. T., N. B. Anthony, and S. R. Lee. 1992. Progeny performance when dams and chicks are fed supplemental zinc. Poult. Sci. 71:1201-1206.

25. Doux Frangosul, Montenegro, RS, Brazil.

26. Cobb-Vantress. 2008. Breeder Management Guide. Cobb Vantress Inc., Arkansas, USA.

27. Zinpro Corp., Eden Prairie, MN. AvailaZMC contained the following amino acids: aspartic acid, 1.29%; glutamic acid, 2.33%; lysine, 0.39%; methionine, 0.15%; threonine, 0.94%; tryptophan, 0.11%; valine, 1.46%; serine, 2.28%; proline, 1.94%; glycine 1.75%; alanine 1.03%; cystine, 0.95%; isoleucine, 0.95%; leucine, 1.56%; tyrosine, 0.54%; phenylalanine, 0.93%; histidine, 0.22% and arginine, 1.31%.

28. AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis, 15th edition. AOAC, Arlington, VA, USA.

29. Anderson K. A. 1999. Minerals—Macro and micro elements in plant tissues by ICPAES. In Analytical techniques for inorganic contaminants. Published AOAC International. pp 110-116.

30. Novikoff, M., and H. S. Gutteridge. 1949. A comparasion of certain methods of estimating shell strength. Poult. Sci. 28:339-343.

31. Fasenko, G. M., F. E. Robinson, B. L. Danforth, and I. Zelter. 2003. Fertility, hatchability, embryo mortality, and chick weight in double versus single yolked broiler breeder eggs. Pages 245-250 in New developments in reproduction and incubation of broiler chickens. Spotted Cow Press, Edmonton, Alberta, Canada.

32. Avicomave, Iracemápolis, SP, Brazil.

33. Cobb-Vantress. 2010. Hatchery Management Guide. Cobb Vantress Inc., Arkansas, USA.

34. Molenaar, R., I. A. M Reijrink, R. Meijerho, H. Van Den Brand. 2008. Relationship between hatchling length and weight on later productive performance in broilers. World's Poul. Sci. J. 64:599-604.

35. Kasuya, E. 2004. Angular transformation – another effect of different sample sizes. Ecol. Res. 19:165-167.

36. SAS Institute Inc. 2009. User’s Guide. Sas Institute Inc., Cary, NC.

37. Little, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216-1231.

38. Long, N. 1996. Model selection in linear mixed effects models using SAS Proc mixed. Ischemia Research, University of California, Berkeley, CA.

39. Wolfinger, R. D. 1996. Heterogeneous variance-covariance structures for repeated measures. J. Agric. Biol. Envir. S. 1:205-230.

40. Kidd, M. T. 2003. A treatise on chicken dam nutrition that impacts on progeny. World's Poult. Sci. J. 59:475-494.

41. Roland, D. A. Sr., D. R. Sloan, and R. H. Harms. 1975. The ability of hens to maintain calcium deposition in the egg shell and egg yolk as the hen ages. Poult. Sci. 54:1720-1723.

42. Naber, E. C. 1979. The effect of nutrition on the composition of the egg. Poult. Sci. 58:518-528.

43. Reis, R. N., S. L. Vieira, P. C. Nascimento, J. E. Peña, R. Barros, C. A. Torres. 2009. Selenium contents of eggs from broiler breeders supplemented with sodium selenite or zinc-L-selenium-methionine. J. Appl. Poult. Res. 18:151-157.

44.Chantiratikul, A., O. Chinrasri, and P. Shantiratikul. 2008. Effect of sodium selenite and zinc-L-selenomethionine on performance and selenium concentrations in eggs of laying hens. Asian-Aust. J. Anim. Sci. 21:1048-1052.

45. Guo, Y. M., R. Yang, J. Yuan, T. L. Ward, and T. M. Fakler. 2002. Effect of AvailaZn and $ZnSO_4$ on laying hen performance and egg quality. Poult. Sci. 81(Suppl. 1):40. (Abstr.).

46. Moreng, R.E., D. Balnave, and D. Zhang. 1992. Dietary zinc methionine effect on eggshell quality of hens drinking saline water. Poultry Sci. 71:1163-1167.

47. Badawy, E. M., B. M. Edrise, and A. M. Al-Wakeel. 1987. The relationship between quality, egg constituents and hatchability of the eggs of Hubbard broiler breeders: A field study. Vet. Med. J. 35:105-115.

48. Vieira, S. L., and E. T. Moran Jr. 1998. Eggs and chicks from broiler breeders of extremely different ages. J. Appl. Poult. Res. 7:372-376.

49. Leeson, S., and J. Summers. 2001. Nutrition of the chicken. 4th ed. Univ. Books, Guelph, Ontario, Canada.

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Table 1. Composition of basal diets supplied to broiler breeders from 22 to 68 wk of age[A]

	Breeder Hens		Males
Ingredients, % as-is	22 to 32 wk	33 to 68 wk	22 to 68
Corn	63.41	64.15	58.93
Soybean meal	22.14	21.40	12.93
Wheat bran	2.00	2.00	23.40
Soybean oil	1.74	1.68	1.00
Limestone	3.84	4.31	1.16
Dicalcium phosphate	1.91	1.64	1.64
Sodium chloride	0.37	0.27	0.33
Sodium bicarbonate	0.13	0.24	0.18
Oyster shells	3.50	3.50	-
Potassium carbonate (98%)	0.27	0.20	0.01
L – Lysine HCl (78%)	0.03	-	-
DL - Methionine (99%)	0.18	0.15	0.08
L - Threonine (98.5%)	0.03	0.01	0.08
Choline chloride	0.10	0.10	0.11
Vitamin premix[B]	0.10	0.10	0.10
Mineral premix[C]	0.05	0.05	-
Male Mineral premix[D]	-	-	0.05
Kaolin[E]	0.20	0.20	-
Total	100.00	100.00	100.00
Calculated nutrient composition, % or as shown			
ME, kcal/kg	2,840	2,840	2,800
Crude protein	15.40 (15.42 ± 1)[F]	15.06 (15.23 ± 1)	13.77 (14.21 ± 1)
Digestible amino acids			
Lys	0.73	0.69	0.55
TSAA	0.63	0.59	0.50
Thr	0.55	0.52	0.51
Ile	0.60	0.59	0.48
Val	0.66	0.64	0.56
Calcium	3.20 (3.33 ± 0.4)	3.30 (3.46 ± 0.3)	0.90 (0.95 ± 0.1)
Non-phytate phosphorus	0.45	0.40	0.45
Total phosphorus	0.64 (0.66 ± 0.1)	0.59 (0.63 ± 0.1)	0.74 (0.70 ± 0.1)
Sodium	0.20	0.20	0.20
DEB, mEq/kg	200.00	200.00	180.00
Choline, mg/kg	1,500	1,500	1,500

[A]Experimental diets were prepared by adding Zn, Mn, and Cu from either $ZnSO_4$, $MnSO_4$, $CuSO_4$, Availa®Zn, Availa®Mn, Availa®Cu.
[B]Vitamin premix provided the following per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 100 IU; vitamin C, 50 mg; vitamin K_3, 6 mg; vitamin B12, 35 µg; thiamine, 3 mg; riboflavin, 15 mg; vitamin B6, 6 mg; niacin, 40 mg; pantothenic acid, 25 mg; folic acid, 4 mg; biotin, 0.3 mg.
[C]Supplied per kilogram of diet: selenium (from sodium selenite), 0.3 mg; iron (from ferrous sulfate), 75 mg; iodine (source = calcium iodate), 1 mg; cobalt (from cobalt sulfate), 1 mg.
[D]Supplied per kilogram of diet: zinc (from zinc sulfate), 100 mg; manganese (from manganese sulfate), 100 mg; copper (from copper sulfate), 10 mg; selenium (from sodium selenite), 0.3 mg; iron (from ferrous sulfate), 75 mg; iodine (source = calcium iodate), 1 mg; cobalt (from cobalt sulfate), 1 mg.
[E]The trace mineral sources replace kaolin (Sericita M-200, Mineração Violani, Colombo, Paraná, Brazil).
[F]Values within parenthesis are analyzed means and standard deviation (SD); n = 12.

Table 2. Calculated[A] and analyzed[B] Zn, Mn, and Cu concentration in the experimental diets and drink water, ppm

	Zn	Mn	Cu
Diet[C]			
CONTROL	157 (146 ± 6)[D]	164 (158 ± 7)	16 (13 ± 1)
ISO	157 (145 ± 5)	164 (155 ± 7)	16 (13 ± 1)
ON TOP	197 (185 ± 6)	204 (202 ± 8)	23(21 ± 1)
Main Ingredients			
Drink water[F]	0.05 ± 0.02	0.03 ± 0.01	0.01 ± 0.001
Corn[G]	54 ± 2	15 ± 1	2 ± 0.5
Soybean meal	81 ± 3	48 ± 2	15 ± 1
Wheat bran	104 ± 4	163 ± 4	11 ±1

[A]Calculated values were obtained using linear feed formulation based on the analyzed Zn, Mn and Cu in all feed ingredients.
[B]Data were performed using inductive coupled plasma atomic emission spectroscopy [29].
[C]CONTROL – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$; ISO – 60 ppm $ZnSO_4$, 60 ppm $MnSO_4$, and 3 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA; ON TOP – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA.
[D]Values within parenthesis are analyzed means and SD; n =12.
[E]Availa®ZMC produced by Zinpro Corporation, Minnesota, USA. Guaranteed composition: 4.00 % of Zn; 4.00 % of Mn; 0.70 % of Cu.
[F]Drink water samples were collected at the nipple drink (Breeder's house). Values are the mean and standard deviation (SD); n = 12.
[G]Corn, soybean meal and wheat bran meal samples were collected from each batch (every 4 wk). Values are the mean and SD; n = 12.

Table 3. Effect of trace mineral treatment and period on breeder performance measures

	Hen-day egg production, %[A]	Hen-housed egg production, %[B]	Total eggs per hen-housed[C]	Settable eggs per hen-housed[D]
Diet[E]				
CONTROL	65.97[F]	62.26	191.79	185.81
ISO	66.41	62.73	193.21	188.46
ON TOP	66.06	62.93	193.82	188.99
SEM	0.52	0.83	2.23	2.24
Period (wk)				
25 – 35	70.25 b[G]	68.47 b	52.72 b	50.59 b
36 – 46	73.87 a	71.94 a	55.40 a	54.21 a
47 – 57	64.20 c	60.64 c	46.69 c	45.86 c
58 – 68	56.26 d	49.52 d	38.13 d	37.10 d
SEM	0.41	0.58	0.45	0.47
Prob.				
Diet	0.8267	0.8472	0.8105	0.5802
Period	0.0001	0.0001	0.0001	0.0001
Diet * Period	0.7603	0.6985	0.6985	0.5787

[a-d]Means within the same column with different superscripts differ ($P \leq 0.05$).
[A]Hen-day egg production, % = (number of egg produced on daily basis / number of birds available in the flock on that day) x 100.
[B]Hen-housed egg production, % = (total number of eggs produced per pen / total number of hens housed) x 100.
[C]Number of total eggs produced from 25 – 68 wks of age divided by the number of hen-housed.
[D]Number of total settable eggs produced from 25 – 68 wks of age divided by the number of hen-housed.
[E]CONTROL – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$; ISO – 60 ppm $ZnSO_4$, 60 ppm $MnSO_4$, and 3 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA; ON TOP – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA.
[F]Means of 200 females per treatment at housing; n = 10 replications of 20 females and 2 males per replicate.
[G]Means of 600 females per period at housing; n = 30 replications of 20 females and 2 males per period.

Table 4. Effect of trace mineral treatment and period on egg shell quality

	Egg weight, g[A]	Specific gravity, g/mL[A]	Relative egg shell weight, %[B]	Egg shell thickness, µm[C]
Diet[D]				
CONTROL	70.37[E]	1.0852	8.96 b	382.09 b
ISO	70.68	1.0852	9.10 a	384.82 a
ON TOP	70.20	1.0858	9.17 a	385.83 a
SEM	0.29	0.00025	0.05	0.97
Period (wk)				
35	65.03 d[F]	1.0871 a	9.39 a	388.77 a
45	69.72 c	1.0861 b	9.33 a	383.47 b
55	72.74 b	1.0843 c	8.82 b	382.84 b
65	74.18 a	1.0841 c	8.76 b	381.23 b
SEM	0.24	0.00027	0.05	1.12
Prob.				
Diet	0.4829	0.1559	0.0164	0.0353
Period	0.0001	0.0001	0.0001	0.0001
Diet * Period	0.0726	0.9376	0.9395	0.8125

[a-d]Means within the same column with different superscripts differ ($P \leq 0.05$).
[A]All settable eggs nest-laid on a single day were measured at 35, 45, 55 and 65 wk of age; n = 20 females per replication.
[B]Egg shell weight, % = (dry egg shell weight / egg weight) x 100; n = 5 eggs per replication per period (35, 45, 55, and 65 wk).
[C]Egg shell thickness was measured at three points on the eggs equatorial zone (settable eggs nest-laid on a single day) after the inner membrane removal; n = 5 eggs per replication per period (35, 45, 55, and 65 wk).
[D]CONTROL – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$; ISO – 60 ppm $ZnSO_4$, 60 ppm $MnSO_4$, and 3 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA; ON TOP – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA.
[E]Means of 200 females per treatment at housing; n = 10 replications of 20 females and 2 males per replicate.
[F]Means of 600 females per period at housing; n = 30 replications of 20 females and 2 males per period.

Table 5. Effect of trace mineral treatment and period on fertility, hatchability, and embryo mortality of broiler breeder[A]

					Embryo Mortality[B]			
	Fertility, %[C]	Hatchability of eggs set, %[D]	Hatchability of fertile, %[E]	Chicks per hen housed[F]	Early dead, %	Middle dead, %	Late dead, %	Pips, %
Diet[G]								
CONTROL	92.62[H]	78.70	88.39 b	147.06 b	3.14 a	0.84	4.63	3.00
ISO	91.08	79.11	90.60 a	149.84 ab	2.08 b	0.51	4.31	2.48
ON TOP	92.18	80.94	90.30 a	153.81 a	2.60 ab	0.71	3.99	2.39
SEM	1.02	1.17	0.92	1.90	0.30	0.15	0.56	0.43
Period (wk)								
45 – 50	93.12 a[I]	84.69 a	92.50 a	42.82 b	1.41 c	0.56 b	3.43 c	2.09
51 – 56	92.04 a	81.15 b	90.72 b	43.98 a	2.04 bc	0.44 b	3.94 bc	2.86
57 – 62	93.57 a	80.21 b	89.04 c	36.75 c	2.69 b	0.68 b	4.98 a	2.59
63 – 68	89.09 b	72.16 c	86.80 d	26.69 d	4.30 a	1.07 a	4.88 ab	2.94
SEM	0.93	1.05	0.84	0.42	0.34	0.17	0.54	0.40
Prob.								
Diet	0.5379	0.1706	0.0659	0.0619	0.0058	0.1152	0.5473	0.3256
Period	0.0006	0.0001	0.0001	0.0001	0.0001	0.0027	0.0206	0.1137
Diet * Period	0.5742	0.3231	0.3169	0.6135	0.9120	0.2079	0.2887	0.7340

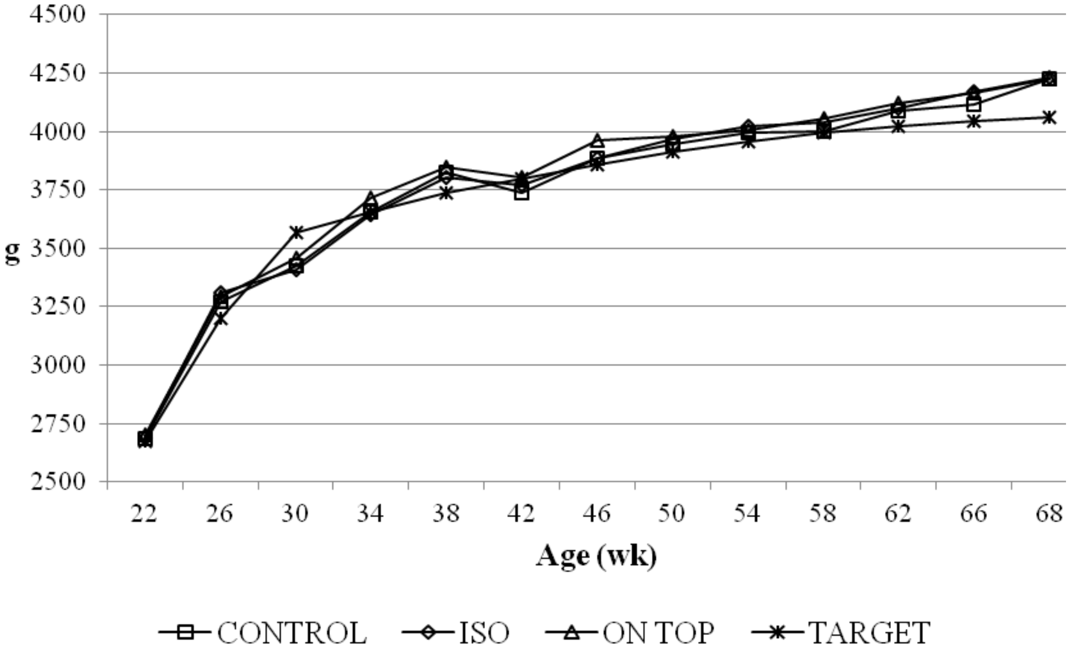
[a-d]Means within the same column with different superscripts differ ($P \leq 0.05$).
[A]Values are the means of 10 replications, each with 20 hens and 2 males at housing; Eggs were incubated and hatched every three wk from 45 to 68 wk; n = 8 incubations.
[B]All data were calculated as a percentage of fertile eggs;
[C]Fertility, % = (number of fertile eggs/ number of total egg set) x 100.
[D]Hatchability of egg set, % = (number of chicks hatched / number of egg set) x 100.
[E]Hatchability of fertile, % = (number of chicks hatched / number of fertile egg set) x 100.
[F]Estimate produced chicks by multiplication of weekly settable eggs per hen-housed per period and hatchability of total eggs set per hatch per period.
[G]CONTROL – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$; ISO – 60 ppm $ZnSO_4$, 60 ppm $MnSO_4$, and 3 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA; ON TOP – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA.
[H]Means of 81 eggs per replicate per incubation; n = 10 replicates.
[I]Means of 2 incubations of 2,430 eggs per incubation per period; n = 30 replicates.

Table 6. Effect of trace mineral treatment and period on hatched chick grading[A]

	Saleable chicks, %[B]	Culls, %[C]	Unhealthy navels, %[D]	Physical abnormalities, %[E]	Hatchling weight, g	Hatchling length, cm
Diet[F]						
CONTROL	84.69[G]	3.44	10.69	1.12	51.28[H]	19.45 b[I]
ISO	85.39	3.56	10.13	0.92	51.35	19.50 ab
ON TOP	87.00	3.19	8.90	0.83	51.18	19.58 a
SEM	1.36	0.57	0.95	0.19	0.21	0.05
Period (wk)						
45 – 50	89.55 a[J]	1.78 b	7.82 b	0.71	49.85 b[K]	19.37 c[L]
51 – 56	86.90 b	4.82 a	7.42 b	0.79	51.62 a	19.51 b
57 – 62	82.93 c	3.95 a	12.14 a	1.08	51.83 a	19.59 a
63 – 68	83.41 c	3.06 ab	12.19 a	1.34	51.78 a	19.58 a
SEM	1.06	0.55	0.94	0.20	0.17	0.03
Prob.						
Diet	0.2444	0.8907	0.1888	0.5520	0.8536	0.0772
Period	0.0001	0.0003	0.0001	0.1342	0.0001	0.0001
Diet * Period	0.9699	0.9169	0.5911	0.4407	0.8193	0.7845

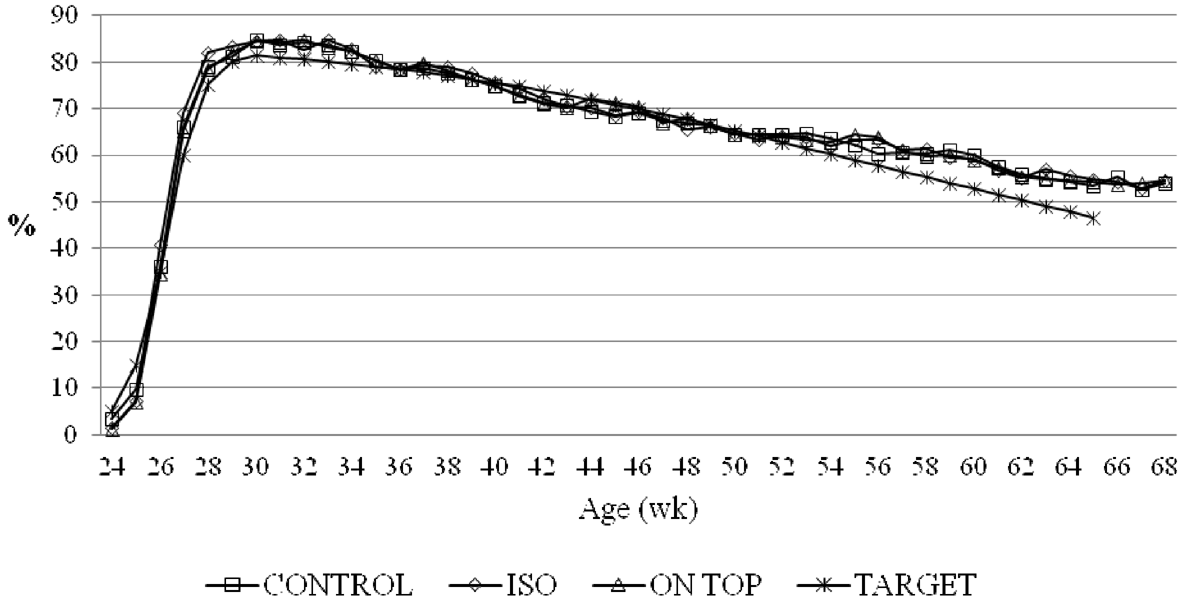
[a-c]Means within the same column with different superscripts differ ($P \leq 0.05$).
[A]All data were calculated as a percentage of hatched chicks; [A]Values are the means of 10 replications, each with 20 hens and 2 males at housing; Eggs were incubated and hatched every three wk from 45 to 68 wk; n = 8 incubations.
[B]High quality chicks.
[C]Weak and red hocks chicks or with some apparent abnormalities.
[D]Chicks at hatching with minor navel conditions: navel buttons (> 2mm) or leaky.
[E]Chicks with some physical problem and difficulty to walk.
[F]CONTROL – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$; ISO – 60 ppm $ZnSO_4$, 60 ppm $MnSO_4$, and 3 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA; ON TOP – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA.
[G]Mean of 81 eggs per replicate per incubation; n = 10 replicates.
[H]Mean of 200 hatched chicks weight; n= 10 replications per treatment.
[I]Mean of 20 chicks divided in 10 males and 10 females per replication per treatment; n = 10 replicates.
[J]Mean of 2 incubations of 2,430 eggs per incubation per period; n = 30 replicates.
[K]Mean of total hatched chicks weight; n = 30 replications per period.
[L]Mean of 2 incubations of 600 males and 600 females per incubation per period; n =30 replicates.

Figure 1. Effect of trace mineral treatment on mean BW of broiler breeder hens



CONTROL – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$; ISO – 60 ppm $ZnSO_4$, 60 ppm $MnSO_4$, and 3 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA; ON TOP – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA.

Figure 2. Effect of trace mineral treatment on hen-day egg production



CONTROL – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$; ISO – 60 ppm $ZnSO_4$, 60 ppm $MnSO_4$, and 3 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA; ON TOP – 100 ppm $ZnSO_4$, 100 ppm $MnSO_4$ and 10 ppm $CuSO_4$ plus 40 ppm ZnAA, 40 ppm MnAA, and 7 ppm CuAA.

CAPÍTULO III[1]

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Development of bone in chick embryos from Cobb 500 breeder hens fed diets supplemented with zinc, manganese and copper from inorganic and amino acid-complexed sources

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ABSTRACT

Sources of Zn, Mn, and Cu (IZMC) as sulphates or as amino acid complexes (OZMC) were used to supplement Cobb 500 breeder hen diets. Experimental treatments consisted of diets supplemented with: 1) 100, 100 and 10 mg/kg of Zn, Mn, and Cu, respectively from IZMC (CONTROL); 2) 60, 60, and 3 mg/kg of Zn, Mn, and Cu, respectively from IZMC plus 40, 40, and 7 mg/kg of Zn, Mn, and Cu, respectively from OZMC (ISO); and 3) a diet with 100, 100 and 10 mg/kg of Zn, Mn, and Cu, respectively from IZMC as in CONTROL plus 40, 40, and 7 mg/kg supplemental Zn, Mn, and Cu from OZMC (ON TOP). Ten replications of 20 females and 2 males were used per treatment. Eggs from breeders at 30, 40, 50 and 60 wk of age were incubated and 5 embryos per replicate were collected at 10 (E10), 14 (E14) and 18 (E18) d of incubation. Midshaft width and calcification were measured for left tibia and femur stained with Alcian Blue and Alizarin Red S. At hatch (DOH) the left tibia of 5 chicks per replicate were sampled for histological evaluation of diaphysis and distal epiphysis. Feeding ISO treatment compared with the CONTROL diet increased the Zn ($P < 0.05$) but not Mn and Cu content of the yolk and albumen blend. At E14, ISO and ON TOP treatments had a trend to increase tibia calcification at the rates of 1.6 and 1%, respectively ($P < 0.1$). The E18 ISO and ON TOP treatments had 2% thicker tibia compared to the CONTROL, regardless of hen age ($P < 0.05$). Also, at E18 calcification of tibia and femur were higher from hens fed ON TOP treatment ($P < 0.05$). The chicks from ISO and ON TOP groups had increased tibia moment of inertia ($P < 0.01$) at DOH. Broiler breeder hens consuming OZMC associated with IZMC produced embryos and hatching chicks with improvements in selected bone mineralization parameters.

Key words: bone, broiler, egg, embryo, mineral

INTRODUCTION

Breeding selection and changes in production efficiency of poultry have resulted in annual improvements in BW gain, feed efficiency, and meat yields (Vieira and Angel, 2012). With these improvements, it became evident that some systems, such as the skeletal system, were not keeping up with the increase in muscle mass (Dibner et al., 2007). Sullivan (1994) estimated that 2 to 5% of the broilers raised are lost annually as a consequence of skeletal problems during the growing and finishing phases due to mortalities and condemnations. Thus, broiler bone development and providing nutrients for a healthy start are important for the breeder industry.

Growth and development of the chick embryo and hatchling are dependent on the nutrient in the fertile egg (Richards, 1997; Uni et al., 2012). It is well documented that the hens' diet mineral concentration and availability are correlated with minerals in the egg for embryo utilization (Kidd, 2003; Dibner et al., 2007). However, trace mineral requirements and availability of trace minerals are not well defined for broiler breeder hens for hatchability and embryo bone development. The importance of trace minerals to the growth and development of embryos is supported by many publications showing that mineral deficiency can reduce hatchability, increase mortality, as well cause skeletal, immune, and cardiovascular system disorders (Kidd, 2003; Dibner et al., 2007).

Feeding diets containing high levels of most minerals in inorganic form to hens has little or no effect on the egg mineral concentration (Naber, 1979; Angel, 2007). In contrast to inorganic trace minerals (ITM), organic trace mineral (OTM) sources and their mixtures have been shown to increase the amount of minerals in the egg components (Hudson et al., 2004; Dobrzanski et al., 2008). Generally, organic trace minerals (OTM) are molecules resulting from an ionic or a covalent bonding between a metal ion and a ligand such as protein or carbohydrate (Vieira, 2008). The use of OTM in animal diets has been increasing because they appear to have greater bioavailability compared to inorganic trace minerals (ITM). This increased availability has been attributed, in part, to an increased solubility and decreased interaction with other nutrients during absorption in the gastrointestinal tract level (Cao et al., 2002). There is a growing interest in the evaluation of organic mineral sources in broiler breeder diets since they have been shown to improve reproductive performance (Kidd, et al., 1992; Virden et al., 2003; Hudson et al., 2004). The objective of this study was to evaluate egg mineral composition and chick embryo bone development when feeding inorganic sulfate and amino acid-complexed sources of Zn, Mn, and Cu in breeder diets.

MATERIALS AND METHODS

Animals

All the procedures in the study were done according to the directives of the Ethics and Research Committee of the Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. Six hundred slow feathering, Cobb 500 broiler breeder hens and sixty Cobb 500 breeder males, 22 wk of age, were obtained from a commercial breeder farm (Doux Frangosul, Montenegro, RS, Brazil). Birds were placed in 30 floor pens (20 females and 2 males) and lighting and feeding throughout the study followed to the Cobb Management Guide (Cobb-Vantress, 2008a).

Experimental Diets

Energy and nutrients daily provided to the breeders followed Cobb recommendations (Cobb-Vantress, 2008b). Two diet phases were used (22 to 32 wk and 33 to 68 wk) for females, but males were fed the same diet throughout the entire study (Table 1). Females were fed separately from males using a separate feeder, which had a head grid constraint. Inorganic sources of Zn, Mn, and Cu (IZMC) (DSM, Nutritional Products, São Paulo, SP, Brazil) as zinc sulfate monohydrate (35% Zn), manganese sulfate monohydrate (31% Mn), and copper sulfate pentahydrate (25% Zn) or organic sources of Zn, Mn, and Cu as amino acid-mineral complex (OZMC) were used (Zinpro Corp., Eden Prairie, MN. Availa®ZMC). Additional information about the amino acid-mineral complex is included in the patent information (Anderson and Abdel-Monem, 2003). The 3 experimental treatments consisted of diets exceeding NRC (1994) requirements containing: 1) 100, 100 and 10 mg/kg of Zn, Mn, and Cu, respectively from IZMC (CONTROL); 2) 60, 60, and 3 mg/kg of Zn, Mn, and Cu, respectively from IZMC plus 40, 40, and 7 mg/kg of Zn, Mn, and Cu, respectively from OZMC (ISO); and 3) a diet with 100, 100 and 10 mg/kg of Zn, Mn, and Cu, respectively from IZMC as in CONTROL plus 40, 40, and 7 mg/kg supplemental Zn, Mn, and Cu from OZMC (ON TOP). Each dietary treatment was replicated 10 times (3 dietary treatments in 30 floor pens). Formulated and analysed nutrient content of the diets are shown in Table 1. Diets samples were collected and analyzed, in duplicate, for CP (988.05), Ca (927.02) (AOAC, 1990), and P (Cavell, 1955).

Trace Mineral Analysis

Basal and experimental diet subsamples were analyzed, in duplicate, for Zn, Mn, and Cu concentration from each feed mix batch (every 4 wk). At 35, 45, 55 and 65 wk of age, yolk and albumen contents from 5 randomly frozen (-18℃) settable eggs per replicate were thawed, mixed, dehydrated in a forced-draft oven at 55 ℃ for 96 h, ground through a 0.5 mm sieve, and sampled.

A representative sample of each diet and eggs (250 mg) were weighed and digested with a mixture of 2 mL of 30% H_2O_2 and 4 mL of 70% HNO_3 for 6 h in a 100 ℃ bath according to AOAC (1999). The digested samp les were analyzed for Zn, Mn, and Cu content using inductively coupled plasma atomic emission spectroscopy (**ICP-AES**) instrument (Spectro Flame Modula E, Kleve, Germany).

Incubation Management

Eggs were collected 4 times daily and a number of 90 eggs per replicate pen were set for incubation at 30, 40, 50 and 60 wk. Only nest-laid eggs that were well shaped, without double yolk, unbroken, uncracked, and with good shell quality were incubated. Eggs were stored during 7 d in a controlled environment room at 18 ℃ and 75% relative humidity, prior to incubation. The settable eggs were placed into replicate trays (n = 30 trays) in a single-stage incubator (Avicomave AV039, Iracemápolis, SP, Brazil) with a 3,600-egg capacity. The incubator was set as 37.5 ^{0}C and 60% relative humidity. On d 18.5, eggs were transferred to the hatcher (Avicomave AV038, Iracemápolis, SP, Brazil), which was set at 36.5 ^{0}C and 65% relative humidity until 21.5 d of incubation.

Embryo Skeleton Staining and Morphometry

One thousand and eight hundred embryos were evaluated for bone skeleton calcification. With eggs collected from breeders at 30, 40, 50, and 60 wks of age, 5 embryos from each of 10 replicates were taken on each of the following embryonic days (E) E10, E14, and E18. Embryonic skeletons were stained with Alcian blue

8GX (C.I. 74240) and Alizarin red S (C.I. 58005) (Vetec Química Fina, Rio de Janeiro, RJ, Brazil) for cartilage and bone ossification, respectively, and cleared in KOH-glycerol. The skeleton staining procedures followed a modification of Ojeda et al. (1970), and Nakane et al. (1999) methods for E10 as follows: 1) Viscera and yolk sac were removed; 2) The remaining embryo was fixed and stained for 2 d at 37℃ in a freshly prepared solution of 95% ethanol (80 mL), 20 mL acetic acid, and 15 mg Alcian blue 8GX; 3) Fixed and stained embryo were dehydrated in 95% ethanol for 7 d with three ethanol changes; 4) Fixed, stained, and dehydrated embryo were stained and macerated for approximately 10 d or more in 2% KOH distilled H_2O solution (bones will start to be divisible at this stage); 5) Stained for 1 d in 0.002% Alizarin red S / 2% KOH distilled H_2O solution; 6) Cleared in glycerine/distilled H_2O solutions of increasing concentration (25, 50 and 75%) of glycerine for 7 d each, to 100% glycerine for storage. For E14, few modifications were done, such as: 1) Skin, adipose tissue, viscera and yolk sac are removed; 2) Fixed and stained as in step (2) for E10; 3) Dehydrated in 95% ethanol for 10 d; three changes of the ethanol; 4) Stained and macerated for approximately 20 d or more in 2% KOH distilled H_2O solution; 5) Stained for 2 d in 0.002% Alizarin red S / 2% KOH distilled H_2O solution; 6) Clearance and storage as in step (6) for E10. For E18: 1) Skin, adipose tissue, viscera and yolk sac are removed; 2) Fixed and stained as in step (2) for E10; 3) Dehydrated in 95% ethanol for 15 d; three changes of the ethanol; 4) Stained and macerated for approximately 30 d or more in 2% KOH distilled H_2O solution; 5) Stained with Alizarin red S, clearance and storage as in step (5) and (6) for E14. The amount of solution in each step above was at least 5 to 8 times more than the volume of embryos being processed. The KOH stain and macerating solution was changed as soon as it started turning yellow.

Macroscopic observation of skeletons was performed under a dissecting stereomicroscope (Labomed CZM6, Labomed, Culver City, CA, USA). The timing of chondrification and calcification of left femur and tibia were documented according to the procedure described by Nakane and Tsudzuki (1999). Chondrification was confirmed by blue color stain with Alcian blue 8GX and calcification by red color with Alizarin red S (Figure 1). Gross morphological measurements were conducted according to the procedure of Blom and Lilja (2004). The amount of calcified bone was measured as the mean length of the red stained part of the bones (femur and tibia) and the amount of cartilage was measured as the mean height of the non-stained parts (i.e. proximal and distal cartilaginous epiphyses). Midshaft width and calcification ((calcified tissue/whole bone)*100) were determined using image analysis software (Image Pro-Plus 4.5.0.29, Media Cybernetics, Inc, Bethesda, Maryland, USA). Images were captured using a digital camera (Olympus E330, Japan) mounted on a stereomicroscope (Labomed CZM6, USA).

Tibia Morphometry and Histomorphometry

Tibias were selected for further analysis because they are known to be the most rapidly growing long bone and, therefore, they are considered more susceptible to mechanical stress (Church and Johnson, 1964). Four hundred and fifty chicks at hatching (DOH) were taken to evaluate tibia bone development. At 30, 40, and 50 wks of breeder age, 5 hatchling chicks from each of 10 replicates were randomly selected and euthanized by cervical dislocation prior to bone collection. Right and left tibias were stripped of any adhering tissue leaving intact cartilage caps on bones. The right tibias were submitted to fat extraction in diethyl ether overnight and subsequently dried. The dried tibias were weighed and their lengths were determined using a digital caliper ruler (Mitutoyo CSX-B, Japan). Bones were later ashed for 10 h at 600℃ in a muffle furnace (Sanchi s, Porto Alegre, RS, Brazil) to determine percent ash ((dry ash weight/dry tibia weight) x 100).

The left tibias were fixed in 10% neutral buffered formalin. Formalin-fixed tibias were decalcified in a 50/50 solution of 20% sodium citrate and 44% formic acid (Vetec Química Fina, Rio de Janeiro, RJ, Brazil) for at least 24 h and then washed with distilled water. Tibias were then dehydrated in graded ethanol solutions. Midshaft and distal epiphysis were cleared in xylene, embedded in paraffin, sectioned at 5 µm on a rotary microtome (Leica, RM2155, Canada), and stained with hematoxylin and eosin. Diaphysis and epiphysis images were captured using a microscope (Olympus BX41, Japan) equipped with a digital camera (Olympus E330, Japan), and the histomorphometric data were obtained using a software image program (Image Pro-Plus 4.5.0.29, Media Cybernetics, Rockville, MD, USA).

To access the influence of diaphyseal geometrical variation on whole bone mechanical properties, inner endosteal and outer periosteal diameters on X and Y-axes of the tibial section were performed using the software image program. Two diaphysis geometric characteristics – moment of inertia and cross-sectional area of the cross-section in relation to the horizontal axis were calculated as shown in Figure 2 (Crenshaw et al., 1981; Ferreti et al., 1993). Ephiphysis growth plate width and area width was evaluated using the proliferative zone, including columnar chondrocytes and the extracellular matrix (Idelevich et al., 2011; Kim et al., 2011). The software image program with semi-automatic function was programmed to measure the growth plate area. The steps for the histomorphometric analysis are outlined in Figure 3. The growth plate appears red-colored compared with the adjacent bone structures (Figure 3A). The region of interest was manually outlined and the software program then automatically displays the area of interest, the growth plate, and calculates the area (Figure 3B) on each slice according to the volume of all red pixels. As the growth plate is not uniform in thickness, 4 vertical (perpendicular to chondro-osseous junction) lines were drawn throughout the growth plate area (Figure 3C), and width was calculated as an average of these 4 measurements.

Statistical Analysis

Data were analyzed using the Repeated Measures Modeling with PROC MIXED procedure of SAS 9.2 (SAS Institute Inc., Cary, NC). The factors time and pen (20 females and 2 males) were considered as fixed and random effects, respectively. The autoregressive covariance structure (AR[1]) was fitted, as it showed best covariance structure based on the Akaike Information Criteria (Little et al., 1998). A consideration was made that measures in all periods had the same variance, means were compared using least squares means comparison and were separated by the PDIFF procedure of SAS when main effect differences or their interaction were detected. Differences were considered significant at $P < 0.05$ and a statistical trend at $P < 0.1$.

RESULTS

Formulated and analyzed diet nutrients were similar (Table 1). The Zn, Mn, and Cu concentrations of basal and experimental diets are presented in Table 2 and exceed the NRC (1994) requirements. A small variation occurred between calculated and analyzed, but the mineral concentration increased from the CONTROL and ISO to ON TOP metal-AA complex diets, as expected.

The data presented in Table 3 show that feeding ISO treatment compared with the CONTROL diet increased the Zn ($P < 0.05$) but not Mn and Cu content of the yolk and albumen blend. Egg mineral composition was affected by period with an initial increase from 35 to 55 wk and decrease between 55 to 65 wk of age ($P < 0.05$). There was no period by diet interaction in any of the parameters evaluated in the current study.

The data presented in Tables 4 show the midshaft width and calcification of tibia and femur in different embryo development stages. At E10, no differences in the tibia midshaft width and calcification between treatments were found (Table 4). Feeding breeder hens with ISO and ON TOP at E14 had a trend ($P < 0.1$) to increase tibia calcification at the rate of 1.6% and 1%, respectively. Following a similar trend the E18 from hens fed ISO and ON TOP treatments had 2.8 and 1.9% thicker tibia ($P < 0.05$); 2.8 and 1.4% thicker femur ($P < 0.1$) than embryos from the CONTROL hens, regardless of hen age. At E18 tibia and femur calcification were greater from hens fed the ON TOP supplementation ($P < 0.05$).

Midshaft width of tibia and femur were affected similarly by period with an increased bone thickness in the breeder age from 30 to 60 wk of age. Bone calcification at E10 decreased with breeder age ($P < 0.01$). However, differences in bone calcification at E18 from early and late breeder ages were less distinct, which could be an indication that chicks from older breeder hens can have a limited skeletal calcification during the early embryonic stages.

Tibia length, weight and ash content at DOH were not affected by the treatments, but were affected by period ($P < 0.01$; Table 5). Based on tibia histology measurements at DOH, the moment of inertia increased ($P < 0.01$) in chicks from hens fed the ISO and ON TOP treatments compared with the CONTROL (Figure 4).

Cross-sectional area of tibia midshaft, distal epiphysis growth plate thickness and area at DOH were affected only by period ($P < 0.05$; data not shown).

DISCUSSION

The main objective in broiler breeder nutrition is to obtain optimum progeny performance. Results of the present study demonstrate that micro mineral source can influence egg mineral content and embryo bone development. Transference of trace minerals from the hen's diet to the egg involves 2 possible routes: the ovary to the yolk and the oviduct to the albumen, shell membrane, and eggshell (Richards, 1997). Mineral transference from the hen into the egg is very important for proper embryo development of crucial and critical organs such as the skeletal system (Kidd, 2003; Dibner et al., 2007).

As minor bone constituents Zn, Mn, and Cu are not usually referred when it comes to evaluate bone mineralization in commercial breeder production; however, their potential impact in the adequate bone mineralization has been demonstrated (Kienholz et al., 1964; Leach, 1976; O'Dell et al., 1961). A component of carbonic anhydrase, Zn plays regulatory roles in bone development (Kienholz et al., 1964; Kidd et al., 1992). Essential for formation of the bone cartilage, Mn is also involved in the formation of proteoglycans (Caskey et al., 1939), which in the epiphyseal bone improves resistance to compression (Leach, 1976). Also important for bone development, Cu has an important role in the cross-linking of collagen and elastin providing tensile strength and elasticity to the bone matrix (O'Dell et al., 1961; Dibner et al., 2007). Besides their essentiality, research with these minerals has shown direct relationship between egg mineral status and progeny responses (Kidd et al., 1992, Hudson et al., 2004).

According to previous research and as well as with the present results, there appears to have 2 possibilities to enrich the egg mineral content: via feeding using OTM and via “in ovo” feeding technology (Uni and Ferket, 2003). Feeding breeder hens with organic Zn, Hudson et al. (2004) observed an increase on the egg Zn content. Results of the current study showed that egg yolk and albumen had 3.2 and 2.2 % more Zn in hens fed the ISO and ON TOP treatments, respectively, compared with the CONTROL. However, the same trend was not observed with Mn and Cu, which could be related with mineral availability on the basal diet, absorption pathways or mineral interaction in the gastrointestinal system. Evaluating the effects of “in ovo” feeding methodology on the embryo nutrient consumption during incubation, Yair and Uni (2011) observed that eggs enriched with minerals at E17 with organic sources of Fe, Zn, Mn, Cu, Ca, and inorganic P (KH_2PO_4) exhibited greater embryo incorporation of Fe, Zn, and Mn between E20 and DOH.

Kidd et al. (1992) observed an increase in the progeny tibia weight when breeders were fed organic Zn and concluded that, either organic Zn was more available from the diet or it was more efficiently transferred from the yolk to the embryo than inorganic Zn improving. It has been recently shown that supplementing organic Zn to broiler chickens consistently increased serum and plasma Ca levels (Feng et al., 2010; Salim et al., 2012). The authors suggested that the results could be attributed to the interactive effects between Zn and Ca metabolism. Present study feeding breeder hens with ISO or ON TOP diets seemed to improve embryo bone mineralization. Furthermore, significant differences were not always consistent on the different evaluated periods, which could be related to the mineral concentration in the diet because the hens feed intake changed over time.

Evaluating the mechanical properties of rat femur diaphysis, Ferreti et al., (1993) observed high correlation coefficients of the moment of inertia and cross-sectional area in the determination of bone strength and stiffness compared to the mean relative wall thickness. The present results show an improvement in the tibia moment inertia suggesting that broiler chicks from hens fed ISO or ON TOP diets have greater strength and stiffness of tibia at DOH compared to the CONTROL group. According with the design proposed in the present study, it is not possible to separate what portion of the response is due to zinc-amino acid complex, manganese-amino acid complex, or copper-amino acid complex. However, it seems clear that the responses observed are due to the replacement of the 3 minerals in the organic source. The use of OZMC in order to supply more available trace minerals is supported by previous researches (Wedekind et al., 1992; Cao et al., 2002). While the importance of amino acids as ligands should not be discounted, the observed effects, however, seemed to be due to the available trace minerals since minimum differences existed for amino acid concentration between diets containing amino acid complexed minerals and the ones having inorganic sources. It seems more reasonable to consider the possibility that synergistic effects of IZMC and OZMC may have actually enhanced the mineral utilization in the mechanisms involved in building structural connective tissue observed in the present study.

To the author's knowledge, the findings in the present study represent the first report of the effects of OZMC on the macro and microscopic chick embryo bone development evaluation. The techniques used can potentially be applied to determine bone modeling in research evaluating the effects of maternal nutrition on the progeny bone development. A better understanding of the levels and the role of each trace mineral in the hen-egg transference and the availability to the embryo is warranted to clarify the distinction between ITM and OTM sources and their interaction in the poultry nutrition.

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REFERENCES

Anderson, M. D., and M. M. Abdel-Monem. 2003. Preparation of metal complexes of amino acids obtained by hydrolysis of soy protein. U.S. Patent No. 6,541,051B2.

Angel, C. R. 2007. Metabolic disorders: Limitation to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. J. Appl. Poult. Res. 16:138-149.

AOAC. 1999. Macro and micro elements in plant tissues by ICPAES. Pages 110-116 in Analytical Techniques for Inorganic Contaminants. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 1990. Official Methods of Analysis, 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Blom, J., and C. Lilja. 2004. A comparative study of growth, skeletal development and eggshell composition in some species of birds. J. Zool. 262:361-369.

Cao, J., P. R. Henry, S. R. Davis, R. J. Cousins, R. D. Miles, R. C. Little, and C. B. Ammerman. 2002. Relative bioavailabity of organic zinc sources based on tissue zinc and metallothionein in chicks fed conventional dietary zinc concentrations. Anim. Feed Sci. Technol. 101:161-170.

Caskey, C., W. Gallup, and L. Norris. 1939. The need for manganese in the bone development of the chick. J. Nutr. 17:407-410

Cavell, A.J., 1955. The spectrophotometric determination of phosphorus in plant material. J. Sci. Food Agric. 6, 479–480.

Church, L. E., and L. C. Johnson. 1964. Growth of long bones in the chicken: Rates of growth in length and diameter of humerus, tibia, and metatarsus. Am. J. Anat. 114:521-538.

Cobb-Vantress. 2008a. Breeder Management Guide. Cobb Vantress Inc., Arkansas, USA. Accessed Feb. 2010. http://www.cobb-vantress.com/contactus/brochures/Breeder_guide_2008.pdf.

Cobb-Vantress. 2008b. Breeder Management Supplement: SF Cobb500. Cobb Vantress Inc., Arkansas, USA. Accessed Feb. 2010. http://www.cobb-vantress.com/contactus/brochures/Cobb500SF_Breeder_Mngmt_Supp_2008.pdf.

Crenshaw, T. D., E. R. Peo, Jr., A. J. Lewis, and B. D. Moser. 1981. Bone strength as a trait for assessing mineralization in swine: a critical review of techniques involved. J. Anim. Sci. 53:827-835.

Dibner, J. J., J. D. Richards, M. L. Kitchell, and M. A. Quiroz. 2007. Metabolic challenges and early bone development. J. Appl. Poult. Res. 16:126-137.

Dobrzanski, Z., K. Mariusz, C. Katarzyna, G. Henryk, and O. Sebastian. 2008. Influence of organic forms of copper, manganese and iron on bioaccumulation of these metals and zinc in laying hens. J. Elementol. 13:309-319.

Feng J., W. Q. Ma, H. H. Niu, X. M. Wu, Y. Wang, and J. Feng. 2010. Effects of zinc glycine chelate on growth, hematological, and immunological characteristics in broilers. Biol. Trace Elem. Res. 133:203-211.

Ferretti, J. L., R. F. Capozza, N. Mondelo, and J. R. Zanchetta. 1993. Interrelationships between densiometric, geometric, and mechanical properties of rat femora: inferences concerning mechanical regulation of bone modeling. J Bone Miner Res. 8:1389-1396.

Hudson, B. P., W. A. Dozier III, J. L. Wilson, J. E. Sander, and T. L. Ward. 2004. Reproductive performance and immune status of caged broiler breeder hens provided diets supplemented with either inorganic or organic source of zinc from hatching to 65 wk of age. J. Appl. Poult. Res. 13:349-359.

Idelevich, A., M. Kerschnitzki, R. Shalar, and E. M. Ornan. 2011. 1,25$(OH)_2D_3$ alters growth plate maturation and bone architecture in young rats with normal renal function. Plos one 6:1-14.

Kidd, M. T. 2003. A treatise on chicken dam nutrition that impacts on progeny. World Poultry Sci. J. 59:475-494.

Kidd, M. T., N. B. Anthony, and S. R. Lee. 1992. Progeny performance when dams and chicks are fed supplemental zinc. Poult. Sci. 71:1201-1206.

Kienholz, E. W., M. L. Sunde, and W. G. Hoekstra. 1964. Influences of dietary zinc, calcium and vitamin D for hens on zinc content of tissues and eggs and on bone composition. Poult. Sci. 43:667-675.

Kim, A., E. Dombi, J. Solomon, E. Fox, F. M. Balis, and B. C. Widemann. 2011. Automated volumetric growth plate measurement using magnetic resonance imaging for monitoring skeletal toxicity in children treated on investigational drug trials. Clin. Cancer Res. 17:5982-5990.

Leach, R. M., Jr. 1976. Metabolism and function of manganese. Pages 235-247 in Trace Elements in Human Health and Disease. Vol. II, A. S. Prasad, ed., Academic Pr., New York.

Little, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216-1231.

Naber, E. C., 1979. The effect of nutrition on the composition of eggs. Poultry Sci. 58:518-528.

Nakane, Y., and M. Tsudzuki. 1999. Development of the skeleton in Japanese quail embryos. Develop. Growth Differ. 41:523-534.

National Research Council. 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

O'Dell, B., B. Harkwick, G. Reynolds, and J. Savage. 1961. Connective tissue defect in the chick resultin from copper deficiency. Proc. Soc. Exp. Biol. Med. 108:402-405.

Ojeda, J. L., E. Barbosa, and G. Bosque. 1970. Selective skeletal staining in whole chicken embryos: A rapid alcian blue technique. Stain Technol. 45:137-138.

Richards, M. P. 1997. Trace mineral metabolism in the avian embryo. Poult. Sci. 76:152-164.

Salim, H. M., H. R. Lee, C. Jo, S. K. Lee, and B. D. Lee. 2012. Effect of sex and dietary organic zinc on growth performance, carcass traits, tissue mineral content, and blood parameters of broiler chickens. Biol. Trace Elem. Res. 147:120-129.

Sullivan, T. W. 1994. Skeletal problems in poulty: Estimated annual cost and description. Poult. Sci. 73:879-882.

Uni, Z., and P. Ferket. 2003. Enhancement of development of oviparous species by in ovo feeding. North Carolina State University, Raleigh, NC and Yissum Research Development Company of the Hebrew Universtity, Jerusalem, Israel, assignees. US Pat. No.6,595,878.

Uni, Z., L. Yadgary, and R. Yair. 2012. Nutritional limitations during poultry embryonic development. J. Appl. Poult. Res. 21:175-184.

Vieira, S. L. 2008. Chelated minerals for poultry. Rev. Bras. Cienc. Avic. 10:73-79.

Vieira, S. L., and C. R. Angel. 2012. Optimizing broiler performance using different amino acid density diets: What are the limits? J. Appl. Poult. Res. 21:149-155.

Virden, W. S., J. B. Yeatman, S. J. Barber, C. D. Zumwalt, T. L. Ward, A. B. Johnson, and M. T. Kidd. 2003. Hen mineral nutrition impacts progeny livability. J. App. Poult. Res. 12:411-416.

Wedekind, K. J., A. E. Hortin, and D. H. Baker. 1992. Methodology for assessing zinc bioavailability: Efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. J. Anim. Sci. 70:178-187.

Yair, R., and Z. Uni. 2011. Content and uptake of minerals in the yolk of broiler embryo during incubation and effect of nutrient enrichment. Poult. Sci. 90:1523-1531.

Table 1. Composition of basal diets supplied to broiler breeders from 22 to 68 week of age[1]

	Breeder Hens		Males
Ingredients, % as-is	22 to 32 wk	33 to 68 wk	22 to 68 wk
Corn	63.41	64.15	58.93
Soybean meal	22.14	21.40	12.93
Wheat bran	2.00	2.00	23.40
Soybean oil	1.74	1.68	1.00
Limestone	3.84	4.31	1.16
Dicalcium phosphate	1.91	1.64	1.64
Sodium chloride	0.37	0.27	0.33
Sodium bicarbonate	0.13	0.24	0.18
Oyster shells	3.50	3.50	-
Potassium carbonate (98%)	0.27	0.20	0.01
L – Lysine HCl (78%)	0.03	-	-
DL - Methionine (99%)	0.18	0.15	0.08
L - Threonine (98.5%)	0.03	0.01	0.08
Choline chloride	0.10	0.10	0.11
Vitamin premix[2]	0.10	0.10	0.10
Mineral premix[3]	0.05	0.05	-
Male Mineral premix[4]	-	-	0.05
Trace minerals/Kaolin[5]	0.20	0.20	-
Total	100.00	100.00	100.00
Calculated nutrient composition, % or as shown			
ME, kcal/kg	2,840	2,840	2,800
Crude protein	15.40 (15.42 ± 1)[6]	15.06 (15.23 ± 1)	13.77 (14.21 ± 1)
Digestible amino acids			
Lys	0.73	0.69	0.55
TSAA	0.63	0.59	0.50
Thr	0.55	0.52	0.51
Ile	0.60	0.59	0.48
Val	0.66	0.64	0.56
Calcium	3.20 (3.33 ± 0.4)	3.30 (3.46 ± 0.3)	0.90 (0.95 ± 0.1)
Non-phytate phosphorus	0.45	0.40	0.45
Total phosphorus	0.64 (0.66 ± 0.1)	0.59 (0.63 ± 0.1)	0.74 (0.70 ± 0.1)
Sodium	0.20	0.20	0.20
Choline, mg/kg	1,500	1,500	1,500

[1]Experimental diets were prepared by adding Zn, Mn, and Cu from either $ZnSO_4$, $MnSO_4$, $CuSO_4$, Availa®Zn, Availa®Mn, Availa®Cu.

[2]Vitamin premix provided the following per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 100 IU; vitamin C, 50 mg; vitamin K_3, 6 mg; vitamin B12, 35 µg; thiamine, 3 mg; riboflavin, 15 mg; vitamin B6, 6 mg; niacin, 40 mg; pantothenic acid, 25 mg; folic acid, 4 mg; biotin, 0.3 mg.

[3]Supplied per kilogram of diet: selenium (from sodium selenite), 0.3 mg; iron (from ferrous sulfate), 75 mg; iodine (source = calcium iodate), 1 mg; cobalt (from cobalt sulfate), 1 mg.

[4]Supplied per kilogram of diet: zinc (from zinc sulphate), 100 mg; manganese (from manganese sulphate), 100 mg; copper (from copper sulphate), 10 mg; selenium (from sodium selenite), 0.3 mg; iron (from ferrous sulfate), 75 mg; iodine (source = calcium iodate), 1 mg; cobalt (from cobalt sulfate), 1 mg.

[5]The trace mineral sources replace kaolin (Sericita M-200, Mineração Violani, Colombo, Paraná, Brazil).

[6]Values within parenthesis are analyzed means and standard deviation (SD); n = 12.

Table 2. Calculated and analyzed[1] Zn, Mn, and Cu concentration in basal and experimental diets, mg/kg

	Zn	Mn	Cu
Basal diet			
Non-supplemented diet	57.42 (41.24 ± 2)	64.21 (49.59 ± 3)	6.13 (3.83 ±1)
Experimental Diets[2]			
CONTROL	157.42 (146.18 ± 6)	164.21 (158.43 ± 7)	16.13 (12.93 ± 1)
ISO	157.42 (145.39 ± 5)	164.21 (154.79 ± 7)	16.13 (13.42 ± 1)
ON TOP	197.42 (184.71 ± 6)	204.21 (202.12 ± 8)	23.13 (21.34 ± 1)

[1]Analysed were done using inductive coupled plasma atomic emission spectroscopy (ICP-AES, AOAC 1999). Values within parenthesis are analyzed means and SD; n =12.
[2]CONTROL – 100 mg/kg $ZnSO_4$, 100 mg/kg $MnSO_4$ and 10 mg/kg $CuSO_4$; ISO – 60 mg/kg $ZnSO_4$, 60 mg/kg $MnSO_4$, and 3 mg/kg $CuSO_4$ plus 40 mg/kg ZnAA, 40 mg/kg MnAA, and 7 mg/kg CuAA; ON TOP – 100 mg/kg $ZnSO_4$, 100 mg/kg $MnSO_4$ and 10 mg/kg $CuSO_4$ plus 40 mg/kg ZnAA, 40 mg/kg MnAA, and 7 mg/kg CuAA.

Table 3. Effect of micro mineral treatments and period on the composition of egg content (yolk and albumen), mg/kg[1]

	Zn	Mn	Cu
Diet[2]			
CONTROL	54.98[b,3]	1.38	2.17
ISO	56.84[a]	1.35	2.22
ON TOP	56.25[ab]	1.48	2.22
SEM	0.693	0.076	0.054
Period (wk)			
35	56.13[a],[4]	0.95[d]	2.09[b]
45	57.43[a]	1.28[c]	2.18[ab]
55	57.60[a]	1.81[a]	2.35[a]
65	52.83[b]	1.59[b]	2.20[ab]
SEM	0.721	0.063	0.070
Prob.			
Diet	0.038	0.308	0.799
Period	0.001	0.001	0.053
Diet * Period	0.975	0.171	0.983

[1]Values are presented on a dry-matter basis.
[2]CONTROL – 100 mg/kg $ZnSO_4$, 100 mg/kg $MnSO_4$ and 10 mg/kg $CuSO_4$; ISO – 60 mg/kg $ZnSO_4$, 60 mg/kg $MnSO_4$, and 3 mg/kg $CuSO_4$ plus 40 mg/kg ZnAA, 40 mg/kg MnAA, and 7 mg/kg CuAA; ON TOP – 100 mg/kg $ZnSO_4$, 100 mg/kg $MnSO_4$ and 10 mg/kg $CuSO_4$ plus 40 mg/kg ZnAA, 40 mg/kg MnAA, and 7 mg/kg CuAA.
[3]Means of 120 eggs; n = 10 (3 settable eggs/replicate/period).
[4]Means of 90 eggs; n = 30 (3 settable eggs/replicate/period).

[a-d] Means with different superscripts within a column signify significant differences (P≤0.05) as a result of a PDIFF

Table 4. Effect of micro mineral treatments and period on calcification of the tibia and femur at different chick embryo days (E)[1]

	Tibia						Femur			
	E10 d		E14 d		E18 d		E14 d		E18 d	
	Midshaft width (mm)	Calcification (%)[2]	Midshaft width (mm)	Calcification (%)	Midshaft width (mm)	Calcification (%)	Midshaft width (mm)	Calcification (%)	Midshaft width (mm)	Calcification (%)
Diet[3]										
CONTROL	0.51[4]	11.12	1.46	69.17^{b}	2.03^{b}	76.74^{b}	1.31	67.83	2.08^{b}	75.90^{b}
ISO	0.52	11.60	1.45	70.28^{a}	2.09^{a}	76.96^{b}	1.30	67.73	2.14^{a}	76.04^{ab}
ON TOP	0.51	11.34	1.47	69.80^{ab}	2.07^{a}	77.59^{a}	1.32	68.68	2.12^{ab}	76.54^{a}
SEM	0.012	0.883	0.023	0.334	0.014	0.189	0.023	0.524	0.023	0.182
Period (wk)										
30	0.48^{b}[5]	16.31^{a}	1.30^{d}	70.33^{b}	1.99^{b}	77.13	1.29^{b}	67.37^{b}	2.01^{c}	75.61^{b}
40	0.51^{a}	13.43^{b}	1.46^{c}	68.90^{c}	2.06^{a}	77.01	1.30^{b}	67.83^{b}	2.13^{b}	76.06^{ab}
50	0.52^{a}	8.95^{c}	1.51^{b}	71.66^{a}	2.09^{a}	77.36	1.31^{ab}	69.00^{a}	2.10^{b}	76.64^{a}
60	0.52^{a}	6.74^{c}	1.58^{a}	68.10^{d}	2.11^{a}	76.88	1.34^{a}	67.99^{b}	2.18^{a}	76.32^{a}
SEM	0.011	0.712	0.021	0.311	0.020	0.228	0.021	0.424	0.021	0.242
Prob.										
Diet	0.620	0.928	0.522	0.067	0.026	0.008	0.677	0.342	0.056	0.049
Period	0.004	0.001	0.001	0.001	0.002	0.528	0.040	0.007	0.001	0.039
Diet * Period	0.577	0.993	0.841	0.275	0.726	0.485	0.903	0.932	0.891	0.874

[1]Midshaft width, bone length and calcification length were measured for embryos stained with Alcian-blue and Alizarin-red S.
[2]Relative calcification was determined using the (calcified length/whole bone length)*100.
[2]CONTROL – 100 mg/kg $ZnSO_4$, 100 mg/kg $MnSO_4$ and 10 mg/kg $CuSO_4$; ISO – 60 mg/kg $ZnSO_4$, 60 mg/kg $MnSO_4$, and 3 mg/kg $CuSO_4$ plus 40 mg/kg ZnAA, 40 mg/kg MnAA, and 7 mg/kg CuAA; ON TOP – 100 mg/kg $ZnSO_4$, 100 mg/kg $MnSO_4$ and 10 mg/kg $CuSO_4$ plus 40 mg/kg ZnAA, 40 mg/kg MnAA, and 7 mg/kg CuAA.
[4]Means of 200 embryos/treatment. n = 10 replicates (5 embryos/replicate/period).
[5]Means of 150 embryos/period. n = 30 replicates (5 embryos/replicate/period).

[a-d]Means within the same column with different superscripts differ significantly ($P \leq 0.05$) and a trend ($P \leq 0.07$).

Table 5. Effect of micro mineral treatments and period on tibia length, weight and ash content at hatch

	Total length (mm)	Dry weight (g)	Ash (%)[1]
Diet[2]			
CONTROL	31.52[3]	0.087	27.45
ISO	31.59	0.088	28.32
ON TOP	31.63	0.090	28.23
SEM	0.151	0.002	0.404
Period (wk)			
30	30.89 [c4]	0.078 [c]	27.28 [b]
40	31.69 [b]	0.090 [b]	29.17 [a]
50	32.16 [a]	0.096 [a]	27.56 [b]
SEM	0.104	0.001	0.393
Prob.			
Diet	0.874	0.156	0.293
Period	0.001	0.001	0.002
Diet * Period	0.973	0.277	0.940

[1]Percentage of ash was determined using (tibia ash weight/defatted tibia dry weight)*100.
[2]CONTROL – 100 mg/kg $ZnSO_4$, 100 mg/kg $MnSO_4$ and 10 mg/kg $CuSO_4$; ISO – 60 mg/kg $ZnSO_4$, 60 mg/kg $MnSO_4$, and 3 mg/kg $CuSO_4$ plus 40 mg/kg ZnAA, 40 mg/kg MnAA, and 7 mg/kg CuAA; ON TOP – 100 mg/kg $ZnSO_4$, 100 mg/kg $MnSO_4$ and 10 mg/kg $CuSO_4$ plus 40 mg/kg ZnAA, 40 mg/kg MnAA, and 7 mg/kg CuAA.
[3]Means of 150 tibia/treatment. n = 10 replicates (5 tibia/replicate/period).
[4]Means of 150 tibia/period. n = 30 replicates (5 tibia/replicate/period).

[a-c]Means within the same column with different superscripts differ significantly (P ≤ 0.05).

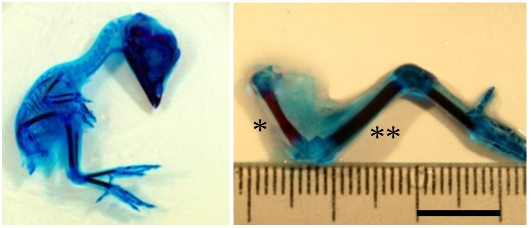
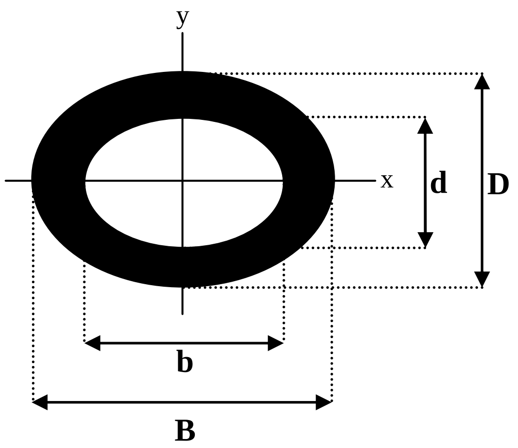


Figure 1. Macroscopic appearance of broiler embryo on embryonic d 14 stained with Alcian-blue and Alizarin-red S showing cartilage and ossified bones, respectively. [*]femur; [**]tibia. Scale bar: 10 mm.



Moment of inertia[1]

$$I_x = 0.491(BD^3 - bd^3)$$

Cross-sectional area[2]

$$A = 0.785\,(BD - bd)$$

Figure 2. Representation of the diaphysis section at tibia midshaft where B and D are outside diameters (mm) of the bone at the point of loading, and b and d are inside diameters (mm) at the same points on Y and X axes. The diameters B and b are diameters perpendicular to the direction of the applied force, while D and d are diameters parallel to the direction of the applied force.

[1]The constant 0.491 equals (π/64) in the calculation of moment of inertia for an ellipse (Crenshaw et al., 1981).

[2]The constant 0.785 equals (π/4) in the calculation of cross-sectional area for an ellipse (Ferretti et al., 1993).

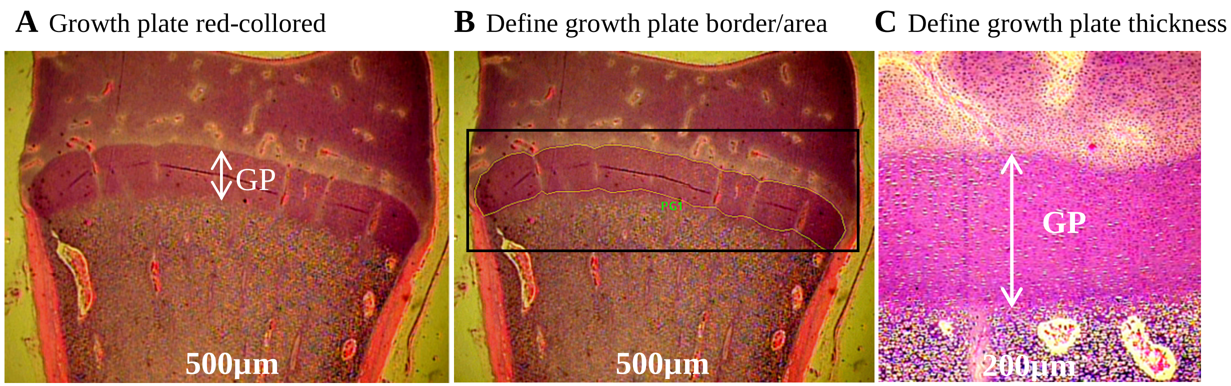


Figure 3. Hematoxylin and eosin-stained sections of the distal epiphysis tibia from broiler on day of hatch. Steps of volumetric growth plate (GP) analysis. A, The growth plate appear redness compared with adjacent bony structures. B, region of interest including the low signal was manually outlined and the software programs then automatically displays the GP and calculates the area (mm^2). C, In each epiphysis zone, 4 vertical lines were drawn throughout the growth plate to calculate the GP width (average of these 4 measurements). Abbreviation: GP-Growth plate.

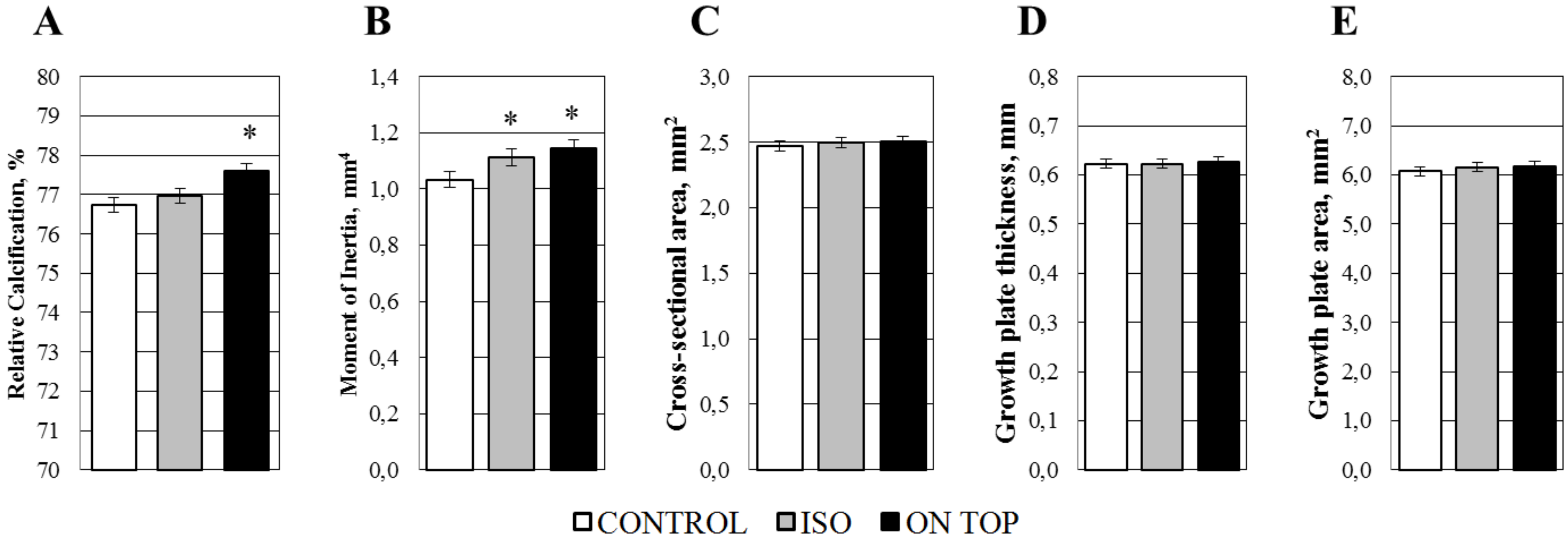


Figure 4. Effect of micro mineral breeder treatments on progeny geometric characteristics and structural properties of the tibia. In panel (A) calcification of broiler embryo on embryonic d 18. The moment of inertia (B) and cross-section area (C) at tibia midshaft on day of hatch. Panel (D) and (E) show the tibia distal epiphysis growth plate thickness and area on day of hatch, respectively. Data are expressed as means ± SE (n=10). *Significant difference (P < 0.01) between breeder hen treatments on progeny. CONTROL – 100 mg/kg $ZnSO_4$, 100 mg/kg $MnSO_4$ and 10 mg/kg $CuSO_4$; ISO – 60 mg/kg $ZnSO_4$, 60 mg/kg $MnSO_4$, and 3 mg/kg $CuSO_4$ plus 40 mg/kg ZnAA, 40 mg/kg MnAA, and 7 mg/kg CuAA; ON TOP – 100 mg/kg $ZnSO_4$, 100 mg/kg $MnSO_4$ and 10 mg/kg $CuSO_4$ plus 40 mg/kg ZnAA, 40 mg/kg MnAA, and 7 mg/kg CuAA.

CAPÍTULO IV

Considerações finais

Ainda que existam certas discrepâncias, resultados promissores têm sido demonstrados no que diz respeito a nutrição de reprodutoras pesadas. Respostas positivas têm se tornado mais constantes quanto aos benefícios da suplementação de microminerais orgânicos para reprodutoras no desenvolvimento da progênie. Uma melhor compreensão, das exigências e do papel de cada micromineral no desenvolvimento da progênie merece ser foco de investigações futuras devido a sua importância e representatividade na cadeia avícola. Neste trabalho, não foi possível isolar os efeitos de cada micromineral avaliado. Talvez a grande limitação para trabalhos com reprodutoras pesadas seja o ciclo longo, número de animais e repetições, infra-estrutura para este tipo de trabalho e consequentemente os custos que são muito superiores comparado a trabalhos com frangos de corte.

A suplementação de microminerais na forma orgânica associado a fontes inorgânicas, parece ser uma importante ferramenta para a produção de pintinhos de um dia de idade de maior qualidade. Além disso, uma alternativa bastante interessante para melhorar a qualidade da casca de ovos tanto na cadeia de produção de ovos incubáveis como nos sistemas de produção da postura comercial.

A deposição de Zn na gema e albúmen foi superior nas aves suplementadas com minerais na forma orgânica. O fato de não ter sido encontrado diferenças para Mn e Cu pode ser devido a termos avaliado a concentração destes minerais apenas no albumen e gema, uma vez que a maior parte destes microminerais ficam depositadas na casca e suas membranas. Neste trabalho, a idéia inicial era avaliarmos a concentração dos microminerais no ovo “in natura”, por isso a casca e membranas não foram incluídas nas amostras, entretanto os limites de detecção ficaram muito baixos e não foi possível definir por este método. Com isso, precisamos desidratar os ovos, moer, homegenizar a amostra e analisar os microminerais. Por este método realizado poderíamos ter trabalhado com análise do ovo inteiro, e consequentemente ter uma maior representatividade da deposição total de minerais no ovo. Para futuros trabalhos, acredito que este seja o melhor método.

As dificuldades do trabalho apresentado, sem dúvida foram as técnicas utilizadas para diafanização e coração de embriões e a histologia óssea no momento pós-eclosão das aves. As principais dificuldades foram associar os dados de literatura de outras espécies animais, diferentes idades e metodologias praticadas. Para cada idade embrionária os métodos, tempos e modo de trabalho são bastante diferentes. No capítulo IV ficou descrita a técnica detalhada para as idades embrionárias avaliadas neste trabalho, caso a avaliação seja feita em idades diferentes alguns detalhes precisam ser ajustados. Para a técnica ser efetiva, uma das coisas mais importantes é a relação de reagentes e material a ser trabalhado. O ideal é que a relação entre reagente e embriões seja de pelo menos 5:1, sendo o ideal 10:1. Quanto maior a relação mais efetiva e rápida é a técnica. Além disso, durante o clareamento dos tecidos moles com cloreto de potássio, embriões de maior idade, necessitam de trocas de reagentes mais periódicas do que embriões mais novos, principalmente nos primeiros dias de clareamento.

Levando em consideração que a idade de abate média de um frango de corte pesado para o mercado interno brasileiro hoje é de 42 dias e frangos tipo “griller” para exportação é de 35 dias, a representatividade do período embrionário na vida destes frangos é de 33 e 37,5%, respectivamente. Com isso, acredito que ganhos em melhora de crescimento neste período são de grande importância para a cadeia avícola mundial. As respostas expressivas encontradas no desenvolvimento esquelético na fase embrionária com a suplementação de minerais orgânicos na dieta das reprodutoras merecem ser foco de futuras investigações para elucidar ainda mais os resultados encontrados.

Dentro do conceito de minerais orgânicos, a dificuldade maior talvez ainda seja conhecer melhor os produtos comerciais disponíveis, como é seu processo fabril, tecnológico e quais têm de fato um efeito positivo no desempenho animal. Na verdade, para cada produto comercial disponível oriundo de processos e tecnologias fabris diferentes, há a necessidade de um novo trabalho e a dificuldade maior ainda é ter acesso a essas informações.

Os principais avanços da tese apresentada talvez sejam vários métodos utilizados para avaliar o desenvolvimento esquelético. As técnicas utilizadas, representam um importante marco na avaliação dos efeitos da nutrição maternal e desenvolvimento esquelético da progênie. Acredito que possa ser um modelo a ser seguido quando se busca avaliar o desenvolvimento esquelético de embriões e pintos pós eclosão frente a novas tecnologias em nutrição animal.

Referências bibliográficas

AAFCO. [**Official publication**]. Atlanta: Association of American Feed Control Officials, 2000.

ABDALLAH, A.G. et al. Effect of removing trace minerals from the diet of hens laying eggs with heavy or light shell weight. **Poultry Science**, Champaign, v. 73, p. 295-301, 1994.

ANGEL, R. Metabolic disorders: Limitations to growth of and mineral deposition into the broiler skeleton after hacth and potential implications for leg problems. **Journal of Applied Poultry Research**, Athens, v.16, p.138-149, 2007.

ASHMEAD, H. D. Comparative intestinal absorption and subsequent metabolism of metal aminoacid quelates and inorganic metal salts. In: AHMEAD H. D. (Ed.). **The roles of aminoacid quelates in animal nutrition**. Westwood, CA: Noyes Publications, 1993.

BAI, S. P. et al. Cloning, sequencing, characterization and expression of divalent metal transporter one in the small intestine of broilers. **Poultry Science**, Champaign, v. 87, p. 768-776, 2008.

BAUMGARTNER, S. et al. Copper deficiency in the laying hen. **Journal of Nutrition**, Rockville, v. 108, p. 804-811, 1978.

BURGER, J. Heavy metals in avian eggshells: Another excretion method. **Journal of Toxicology and Environmental Health**, London, UK, v. 41, p. 207-220, 1994.

BURLEY, R. W.; VADEHRA, D. V. **The avian egg chemistry and biology**. New York: John Wiley and Sons, 1989.

CABELL, C. A.; EARLE, I. P. Additive effect on calcium and phosphorus on utilization of dietary zinc. **Journal of Animal Science**, Savoy, v. 24, p. 800-806, 1965.

CAO, J. et al. Relative bioavailability of organic zinc sourcer based on tissue zinc and metallothionein in chicks fed conventional dietary zinc concentrations. **Animal Feed Science and Technology**, Amsterdam, v. 101, p. 161-170, 2002.

CARLTON, W.; HENDERSON, W. Chickens fed a copper-deficient diet supplemented with ascorbic acid, reserpine, and diethylstilbestrol. **Journal of Nutrition**, Rockville, v. 85, p. 67-72, 1965.

CASKEY, C.; GALLUP, W.; NORRIS, L. The need for manganese in the bone development of the chick. **Journal of Nutrition**, Rockville, v. 17, p. 407-410, 1939.

COUNCIL directive 1999/29/EC of 22 april 1999 on the undesirable substances and products in Animal Nutrition. **Official Journal of the European Communities**, [Luxembourg], L115, p. 32-46, 1999.

DECUYPERE, E. et al. The use of high zinc diets as forced moting method: effect of Zn uptake and egg Zn content. **Archiv für Geflügelkunde**, Stuttgart, v. 52, p. 245-251, 1988.

DEWAR, W. A.; TEAGUE, P. W.; DOWNIE, J. N. The transfer of minerals from the egg to the chick embryo from the 5th to the 18th daysof incubation. **British Poultry Science**, London, v.15, p.119-129, 1974.

DIBNER, J.J. et al. Metabolic challenges and early bone development. **Journal of Applied Poultry Research**, Athens, v.16, p.126-137, 2007.

ELVEHJEM, C. A.; KEMMERER, A. R.; HART, E. B. The effect of the diet of the hen on the iron and copper content of the egg. **Journal of Biological Chemistry**, Baltimore, v. 85, p. 89-96, 1929.

ENSMINGER, M. E.; OLDFIELD, J. E. **Feeds & Nutrition**. 2nd ed. California: Ensminger Publishing Company, 1990. 500 p.

EVANS, G.; GRACE, C.; VOTAVA, H. Proposed mechanism of zinc absorption in the rat. **American Journal of Physiology**, Bethesda, v. 228, p. 501-505, 1975.

FLEET, J. C.; MCCORMICK, C. C. The ontogeny and induction by zinc of hepatic chick embryo metallothionein. **Proceedings of the Society for Experimental Biology and Medicine**, Malden, v. 188, p. 52-60, 1988.

FRELINGER, J.A. Maternally derived transferrin in pigeon squabs. **Science**, New York, v. 171, p. 1260-1261, 1971.

GALLUP, W. D.; NORRIS, L. C. Assimilation and storage of manganese in the developing embryo and growing chick. **Poultry Science**, Champaign, v. 18, p. 99-105, 1939.

GARRICK, M.D. et al. A mammalian transporter for multiple metals. **Biometals**, Oxford, v. 16, p. 41-54, 2003.

GEORGIEVSKI, V. I.; ANNEKOV, B. N.; SAMOKHIN, V. T. **Mineral nutrition of animals**. London: Butterworth, 1982. p. 11-56.

GUENTHNER, E.; CARLSON, C. W.; EMERICK, R. J. Copper salts for growth stimulation and allevation of aortic rupture losses in turkeys. **Poultry Science**, Champaign, v. 57, p. 1313-1324, 1978.

HARMS, R. H.; AMMERMAN, C. B.; WALDROUP, P. The effect of supplemental phosphorus in the breeder diet upon hatchability of eggs and bone composition of the chicks. **Poultry Science**, Champaign, v. 43, p. 209-212, 1964.

HEMPE, J. M.; COUSINS, R. J. Effect of EDTA and zinc-methionine complex on zinc absorption by rat intestine. **Journal of Nutrition**, Rockville, v. 119, p. 1179-1187, 1989.

HILL, C. H.; MATRONE, G. Chemical parameters in the study of in vivo and in vitro interactions of transition elements. **Federation Proceedings**, Washington, v. 29, p. 1474-1481, 1970.

HILL, R. **Trace elements in man and animals**. Batimore: University Park Press, 1974.

HUDSON, B. P. et al. Breeder age and zinc source in broiler breeder hen diets on progeny charateristics at hatching. **Journal of Applied Poultry Research**, Athens, v.13, p. 55-64, 2004a.

HUDSON, B. P. et al. Reproductive performance and immune status of caged broiler breeder hens provided dites supplemented with either inorganic or organic sourcer of zinc from hatching to 65 wk of age. **Journal of Applied Poultry Research**, Athens, v. 13, p. 349-359, 2004b.

KIDD, M. T.; ANTHONY, N. B.; LEE, S. R. Progeny performance when dams and chicks are fed supplemental zinc. **Poultry Science**, Champaign, v. 71, p. 1201-1206, 1992.

KIDD, M. T. et al. Effect of a supplemental zinc in either a corn soy beans or a milo and corn soybean meal diet on the performance of young broiler breeders and their progeny. **Poultry Science**, Champaign, v. 72, p. 1492-1499, 1993.

KIDD, M. T. et al. Boold clearance of Escherichia coli and evaluation of mononuclear-phagocytic system as influenced by supplemental dietary zinc methionine in young turkeys. **Poultry Science**, Champaign, v. 73, p. 1381-1389, 1994.

KIDD, M. T. et al. Turkey hen zinc source affects progeny immunity and disease resistance. **Journal Applied of Poultry Research**, Athens, v. 9, p. 414-423, 2000.

KIIDD, M. T. A treatise on chicken dam nutrition that impacts on progeny. **World's Poultry Science Journal**, Columbus, v. 59, p. 475-494, 2003.

KIENHOLZ, E. W. et al. Effects of zinc deficiency in the diets of hens. **Journal fo Nutrition**, Rockville, v. 75, p. 211-213, 1961.

KIENHOLZ, E. W.; SUNDE, M. L.; HOEKSTRA, W. G. Influences of dietary zinc, calcium and vitamin D for hens on zinc content of tissues and eggs and on bone composition. **Poultry Science**, Champaign, v. 43, p. 667-675, 1964.

KIMURA, I. Developmental change in microheterogeneity of serum transferrin of chickens. **Development, Growth & Differentiation**, Tokyo, v. 15, p. 531-535, 1983.

KRAM, D.; KLEIN, N. W. Serum protein synthesis in the early chick embryo. **Developmental Biology**, London, v. 52, p. 300-309, 1976.

LAMBSON, R. O. An electron microscopic study of the entodermal cells of the yolk sac of the chick during incubation and after hatching. **American Journal of Anatomy**, New York, v. 129, p. 1-20, 1970.

LATSHAW, J. D. Natural and selenite selenium in the hen and the egg. **Journal fo Nutrition**, Rockville, v. 105, p. 32-37, 1975.

LATSHAW, J. D.; BIGGERT, M. D. Incorporation of selenium into egg proteins after feeding selenomethionine or sodium selenite. **Poultry Science**, Champaign, v. 60, p. 1309-1313, 1981.

LEACH JR., R. M. Metabolism and function of manganese. In: PRASAD, A. S. (Ed.). **Trace elements in human health and disease**. New York: Academic Press, 1976.

LEESON, S.; SUMMERS, J. **Nutrition of the chicken**. 4. ed. Guelph: Univ. Books, 2001.

LOWEL, J. A.; WISEMAN, J.; COLE, D. J. A. Absorption and retention of zinc when administered as an amino-acid chelate in the dog. **Journal of Nutrition**, Rockville, v. 124, p. 2572-2574, 1994.

LUSCOMBE, N. M. Review: an overview of the structures of protein-DNA complexes. **Genome Biology**, London, v. 1, p. 001.01-001.10, 2000.

MAS, A.; AROLA, L. Cadmium and lead toxicity effects on zinc, copper, nickel and iron distribution in the developing chick embryo. **Comparative Biochemistry and Physiology**, London, v. 80, p. 185-188, 1985.

MCCORMICK, C. C.; CUNNINGHAM, D. L. High dietary zinc and fasting as methods of forced resting: a performance comparison. **Poultry Science**, Champaign, v. 63, p. 1201-1206, 1984.

MCFARLANE, W. D.; MILNE, H. I. Iron and copper metabolism in the developing chick embryo. **Journal of Biological Chemistry**, Baltimore, v. 107, p. 309-319, 1934.

MELLOR, D. Historical backgrouns and fundamental concepts “of chelation”. In: DWYER, F; MELLOR, D (Ed.). **Chelating agents and metal chelates**. New York: Academic Press, 1964.

MITCHELL, M. A.; CARLISLE, A. J. Plasma zinc as an index of vitellogenin production and reproductive status in the domestic fowl. **Comparative Biochemistry and Physiology**, London, v. 100, p. 719-724, 1991.

MOBBS, I. G.; MCMILLIAN, D. B. Structure of the endodermal epithelium of the chick yolk sac during early stages of development. **American Journal of Anatomy**, New York, v. 155, p. 287-310, 1979.

NABER, E. C. The effect of nutrition on the compositition of eggs. **Poultry Science**, Champaign, v. 58, p. 518-528, 1979.

NATIONAL RESEARCH COUNCIL (NRC). **Nutrient requirements of poultry**, 9th rev. ed. Washington: National Academy Press, 1994.

O’DELL, B. et al. Connective tissue defect in the chick resultin from copper deficiency. **Proceedings of the Society for Experimental Biology and Medicine**, Malden, v. 108, p. 402-405, 1961.

O’SULLIVAN, N. P.; DUNNINGTON, E. A.; SIEGEL, P. B. Relationships among age of dam, egg component, embryo lipid transfer and hatchability of broiler breeder eggs. **Poultry Science**, Champaign, v. 70, p. 2180-2185, 1991.

PACKARD, M. J.; PACKARD, G. C. Patterns of mobilization of calcium, magnesium, and phosphorus by embryonic yellow-headed blackbirds (*Xanthocephalus xanthocephalus*). **Journal of Comparative Physiology B**, New York, v. 160, p. 649-654, 1991.

PAIK, I. K. et al. Effects of supplementary cooper-chelate on the performance and cholesterol level in plasma and breast muscle of broiler chickens. **Journal of Animal Science**, Savoy, v. 12, p. 794-798, 1999.

PALMITER, R. D. Regulation of protein synthesis in the chick oviduct. I. Independent regulation of ovalbumin, conalbumin, ovomucoid, and lysozyme induction. **Journal of Biological Chemistry**, Baltimore, v. 247, p. 6450-6461, 1972.

PATTEN, B. M.; CARLSON, B. M. **Foundations of embryology**. New York: McGraw-Hill, 1974.

RAMSAY, W. N. M. Iron metabolism and haemoglobin formation in the embryonated hen egg. **Biochemistry Journal**, Washington, v. 49, p. 494-499, 1951.

REBEL, J. M. J. et al. Vitamin and trace mineral content in feed of breeders and their progeny: effects of growth, feed conversion and severity of malabsorption syndrome of broilers. **British Poultry Science**, London, v. 45, p. 201-209, 2004.

REDDY, A. B.; DWIVED J. N.; ASHMEAD, A. D. Mineral chelation generates profit. **Misset World Poultry**, Doetinchem, v. 8, p. 13-15, 1992.

RICHARDS, M.P.; WEINLAND, B.T. Hepatic zinc, copper, and iron in the developing turkey embryo and newly hatched poult. **Biological Trace Element Research**, London, v. 7, p. 269-283, 1985.

RICHARDS, M. P.; STEELE, N. C. Trace element metabolism in the developing avian embryo: A review. **Journal of Experimental Zoology**, New York, v. 1, p. 39-51, 1987.

RICHARDS, M. P. Influence of egg production on zinc, copper, and iron metabolism in the turkey hen (*Meleagris gallopavo*). **Comparative Biochemistry and Physiology**, London, v. 93, p. 811-817, 1989.

RICHARDS, M. P.; STOCK, M. K.; METCALFE. J. Effects of brief hypoxia and hyperoxia on tissue trace element levels in the developing chick embryo. **Magnesium and Trace Elements**, Basel, v. 10, p. 305-320, 1991.

RICHARDS, M. P.; PACKARD, M. J. Mineral metabolism in avian embryos. **Poultry and Avian Biology Reviews**, London, v. 7, p. 143-161, 1996.

RICHARDS, M. P. Trace mineral metabolism in the avian embryo. **Poultry Science**, Champaign, v. 76, p. 152-164, 1997.

ROMANOFF, A.L. **The avian embryo**: structural an functional development. New York: Macmillan Co., 1960.

ROMANOFF, A. L. **Biochemistry of the avian embryo. A quantitative analysis of prenatal development**. New York: John Wiley and Sons, 1967.

SANDROCK, B.; KERN, S. R.; BRYAN, S. E. The movement of zinc and copper from the fertilized egg into metallothionein-like proteins in developing chick hepatic tissue. **Biological Trace Element Research**, London, v. 5, p. 503-515, 1983.

SANDSTROM, B. Dose dependence of zinc and magnesium absorption in man. **Proceedings of Nutrition Society**, Wallingford, v. 51, p. 211-218, 1992.

SAVAGE, J. Trace minerals and avian reproduction. **Federation Proceedings**, Washington, v. 27, p. 927-931, 1968.

SCHEIDELER, S.E. Interaction of dietary calcium, manganese, and manganese source (Mn oxide or Mn methionine complex) on chick performance and manganese utilization. **Biological Trace Element Research**, London, v. 29, p. 217-228, 1991.

SHENSTONE, F.S. **The gross composition, chemistry, and physical-chemical basis organization of the yolk and white. A study of the hen's egg**. Edinburgh: Oliver and Boyd, 1968.

SHIPPEE, R.L. et al. High dietary zinc or magnesium as forced-resting agents for laying hens. **Poultry Science**, Champaign, v. 58, p. 949-954, 1979.

STADELMAN, W. J.; PRATT, D. E. Factors influencing composition of the hen's egg. **World's Poultry Science Journal**, Columbus, v. 45, p. 247-266, 1989.

STAHL, J. L.; COOK, M. E.; GREGER, J. L. Zinc, iron and copper contents of eggs from hens fed varying levels of zinc. **Journal of Food Composition and Analysis**, San Diego, v. 1, p. 309-315, 1988.

STEVENSON, M. H.; PEARCE, J.; JACKSON, N. J. The effects of dietary intake and of dietary concentration of copper sulphate on the laying domestic fowl: effects on laying performance and tissue mineral contents. **British Poultry Science**, London, v. 24, p. 327-355, 1983.

SUYAMA, K.; FUKAZAWA, Y.; UMETSU, Y. A new biomaterial, hen egg shell membrane, to eliminate heavy metal ions from their dilute waste solution. **Applied Biochemistry and Biotechnology**, Clifton, v. 45/46, p. 871-879, 1994.

SWANSON, C. A. Comparative utilization of selenite, selenomethionine, and selenized yeast by the laying hen. **Nutrition Research**, Los Angeles, v. 7, p. 529-537, 1987.

TAMIM, N.; ANGEL, R. Phytate phosphorous hydrolysis as influenced by dietary calcium and micro-mineral source in broiler diets. **Journal of Agricultural and Food Chemestry**, Washington, v. 51, p. 4687-4693, 2003.

TRIYUWANTA, C. I.; LETERRIER, C.; NYS, Y. Dietary phosphorus and food allowance of dwarf breeders affect reproductive performance of hens and bone development of their progeny. **British Poultry Science**, London, v. 33, p. 363-379, 1992.

UNDERWOOD, E. J. **Trace elements in human and animal nutrition**. New York: Academic Press, 1977.

UNDERWOOD, E. J.; SUTTLE, N. F. **The mineral nutrition of livestock**. London: CABI Publishing, 2001.

UNI, Z.; YADGARY, L.; YAIR, R. Nutritional limitations during poultry embryonic development. **Journal of Applied Poultry Research**, Athens, v. 21, p. 175-184, 2012.

UNIÃO BRASILEIRA DE AVIBULTURA (UBA). **Relatório anual 2010/2011 da União Brasileira de Avicultura**. São Paulo, 2012. 72 p.

VIEIRA, S. L. Chelated minerals for poultry. **Brazilian Journal of Poultry Science**, Campinas, v. 10, p. 73-79, 2008.

VIEIRA, S. L.; MORAN JR., E. T. Eggs and chicks from broiler breeders of extremely diferente age. **Journal Applied of Poultry Research**, Athens, v. 7, p. 372-376, 1998.

VIRDEN, W. S. et al. Hen mineral nutrition impacts progeny livability. **Journal Applied of Poultry Research,** Athens, v. 12, p. 411-416, 2003.

VOHRA, P.; GRAY, G. A.; KRATZER, F. H. Phytic acid-metal complexes. **Proceedings of the Society of Experimental Biology and Medicine**, New York, v. 120, p. 447-449, 1965.

WEDEKIND, K. J.; HORTIN, A. E.; BAKER, D. H. Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. **Journal of Animal Science**, Savoy, v. 70, p. 178-187, 1992.

WEDEKIND, K. J. et al. Bioavailability of zinc from inorganic and organic sources for pigs fed corn-soybean meal diets. **Journal of Animal Science**, Savoy, v. 72, p. 2681-2689, 1994.

WILLIAMS, J. Serum proteins and the livetins of hen's-egg yolk. **Biochemistry Journal**, Washington, v. 83, p. 346-355, 1962.

YAIR, R.; UNI, Z. Content and uptake of minerals in the yolk of broiler embryo during incubation and effect of nutrient enrichment. **Poultry Science**, Champaign, v. 90, p. 1523-1531, 2011.

YOUNG, M. F.; KLEIN, N. W. Synthesis of serum proteins by cultures of chick embryo yolk sac endodermal cells. **Developmental Biology**, London, v. 100, p. 50-58, 1983.

APÊNDICES

Apêndice 1. Normas para publicação de artigos no periódico Journal of Applied Poultry Research

Journal of Applied Poultry Research
Instructions to Authors
Editorial Policies and Procedures

The mission of *Journal of Applied Poultry Research* (JAPR) is to provide practical, reliable, and timely information to those whose livelihoods are derived from the commercial production of poultry and those whose research benefits this sector; address topics of near-term application based on appropriately designed studies and critical observations; encourage scientific approaches to practical problem solving; and present information comprehensible to a broad readership.

By submission of a manuscript, the authors guarantee to the journal that the work described has not been published before (except in the form of an abstract or as part of a published lecture, review, thesis, or dissertation); that it is not under consideration for publication elsewhere; and that its publication has been approved by all coauthors, if any, as well as by the responsible authorities at the institute where the work has been carried out. Appropriate identification of previously published preliminary reports should be provided in a title page footnote. Translations of an article into other languages for publication require approval by the editor-in-chief. Opinions or views expressed in papers published by JAPR are those of the authors and do not necessarily represent the opinion of the Poultry Science Association (PSA) or the editor-in-chief.

Before manuscripts are submitted, authors should have them read critically by others well versed in English to facilitate review; all co-authors should approve the manuscript before its submission to the journal.

Contact Information for Journal Staff

For information on the scientific content of the journal, contact the editor-in-chief, Dr. Jesse Grimes, North Carolina State University, Department of Poultry Science, Box 7608, Raleigh, NC 27695 (e-mail: jesse_grimes@ncsu.edu). For assistance with Manuscript Central, manuscript submission and copyright forms, or page charge and offprint orders, contact the editorial assistant, Jeremy Holzner, PSA, 2441 Village Green Place, Champaign, IL 61822 (FAX: 217-378-4083; jeremyh@assochq.org).

For other information or to submit a paper, contact the editorial department, PSA, 2441 Village Green Place, Champaign, IL 61822; (telephone: 217-356-7641; FAX: 217-378-4083; journals@assochq.org).

Care and Use of Animals

Authors must make it clear that experiments were conducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and laboratory techniques. Experiments shall be conducted in accordance with the principles and specific guidelines presented in *Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*, 1st revised edition, 1999 (Association Headquarters, 2441 Village Green Place, Champaign, IL 61822); and, if applicable, *Guide for the Care and Use of Laboratory Animals* (United States Department of Human Health and Services, National Institutes of Health, Publication Number ISBN 0-309-05377-3, 1996); or *Guide to the Care and Use of Experimental Animals*, 2nd ed., Vol.

1, 1993 (Canadian Council on Animal Care). Methods of killing experimental animals must be described in the text. In describingsurgical procedures, the type and dosage of the anesthetic agent must be specified. Intra-abdominal or intra-thoracic invasive surgery requires anesthesia. This includes caponization.

The editor-in-chief of JAPR may refuse to publish manuscripts that are not compatible with these guides. If rejected solely on that basis, however, the paper may be resubmitted for reconsideration when accompanied by a written verification that a committee on animal care in research has approved the experimental design and procedures involved.

Types of Articles

Research Reports. Most papers published in JAPR are research reports. The journal emphasizes the importance of good scientific writing and clarity in presentation of the concepts, apparatus, and sufficient background information that would be required for thorough understanding by scientists in other disciplines. The results of experiments published in JAPR must be replicated, either by replicating treatments within experiments or by repeating experiments.

In addition to research reports, other types of papers appear in the journal:

Field Reports. Field reports will be published when adequate background is available and conclusions can be supported by quantifiable laboratory or diagnostic results. The manuscript should follow the format outlined in the Style and Form. It should include a section titled Field Report in which the observations are explained and discussed under subheadings of Materials and Methods and Results and Discussion. Authors are encouraged to include subheadings for all major areas in this section.

Review Articles. Articles submitted to this section may cover new developments in a field, describe the evolution of a currently accepted management practice, propose changes in management based on current research, or describe procedures. Clear distinctions should be made between firmly established practices and unresolved questions. Articles should begin with a concise description of the topic, followed by a critical evaluation of the important references. Review articles, whether solicited or unsolicited, will be subject to a stringent review process. Review articles should follow the general format outlined in the Style and Form when appropriate and include brief subheadings to separate main ideas. The title page should use the appropriate format and include a summary and statement of primary audience. Review articles may include tables, figures, and photographs. A Conclusions and Applications section should be included in most cases.

The use of copyrighted materials must be by permission of the copyright holders. Authors are responsible for obtaining copyright permissions and sending them to the managing editor.

Symposium and Workshop Articles. Manuscripts presented at the annual meeting as part of a symposium or workshop may be submitted with prior agreement by the editor-in-chief. These submissions will be subject to peer review and may be accepted or rejected in the same manner as other submissions. The format may be similar to reviews, research reports, or field reports, as outlined in the Style and Form.

Letters and Commentaries. The journal accepts letters, book reviews, and other free-form communications (used to correct errors, provide clarification, or offer other points of view on pertinent issues). Submissions may be edited in consultation with the author.

SUBMISSION OF MANUSCRIPTS

Authors should submit their papers online to our Web-based submission and review system (http://mc.manuscriptcentral.com/psa). Detailed instructions for submitting electronically are provided online at that site. Authors who are unable to submit online should contact the editorial office (jeremyh@assochq.org) for assistance.

Copyright Agreement

When a manuscript is accepted for publication, the authors agree to transfer copyright to the publisher, that the manuscript will not be published elsewhere in any language without the consent of the copyright holders, that written permission of the copyright holder has been obtained by the authors for material used from other copyrighted sources (including tables, graphs, figures, and illustrations), and that any costs associated with obtaining this permission are the authors' responsibility.

The Manuscript Submission and Copyright Release Form (available on the JAPR Web site: http:// japr.fass.org/misc/ifora.dtl) must be completed and filed with the editorial office for each paper submitted; faxed copies are acceptable. The copyright agreement is included in the Manuscript Submission and Copyright Release form and must be completed by all authors before publication can proceed. The corresponding author is responsible for obtaining the signatures of co-authors. Authors who are not permitted to release copyright, such as federal employees, must still sign and return the form with a statement of the reason for not releasing the copyright.

REVIEW OF MANUSCRIPTS

The journal uses a two-stage review process. All manuscripts will first receive a preliminary review to ensure appropriateness for the journal. The second review will be a more detailed scrutiny by individuals knowledgeable in the specific subject area of the paper. Additional examination of the manuscript will be made by the editors. The review process will be stringent. Names of authors will be made known to reviewers; reviewers may contact the authors directly with questions, suggestions, and comments if such contact will improve the paper or streamline the review process. The subject editors will handle all initial correspondence with authors during the review process; the editor-in-chief will notify the author of the final decision to accept or reject.

PRODUCTION OF PROOFS

Accepted manuscripts are forwarded to the editorial department for preparation for typesetting. At this point, a technical editor may contact the authors for missing information or table or figure revisions.

The manuscript is then typeset, figures are reproduced, and author proofs are prepared.

Proofs

Author proofs of all manuscripts will be sent to the corresponding author indicated on the title page of the manuscript. Proofs should be read carefully, because the responsibility for proofreading is with the authors.

Corrections to the proof should be made neatly and clearly in the margins of the proof. Galley proofs should be faxed (217-378-4083) to PSA headquarters. Proofs should be corrected and returned within 3 working days.

Editor queries appear in the text, within brackets and in boldface type. Queries should be answered on the galley proofs; failure to do so may delay publication.

Publication Charges and Offprints

Two options are available for the publication of articles in this journal: conventional page charges and Open Access (**OA**).

Conventional Page Charges. The current charge for publication is $60 per printed page (or fraction thereof) in the journal if at least one author is a current professional member of PSA. If no author is a member of PSA, the publication charge is $85 per journal page. ***OA.*** For authors who wish to publish their papers OA (freely available without subscription when the issue is posted online), authors will pay the OA fee when proofs are returned to the editorial office.

Charges for OA are $2,400 if at least one author is a current professional member of PSA; the charge is $3,100 when no author is a professional member.

Offprints and Color Charges. Offprints may be ordered at an additional charge. Authors who submit articles containing color illustrations are responsible for paying the additional charge for color printing, including the printing of any reprints they order, and must agree in writing prior to publication to pay the additional charges (http://japr.fass.org/misc/JAPR_ColorChargeAgreement.pdf). When the galley proof is sent, the author is asked to complete an offprint order indicating the number of offprints desired and the name of the institution, agency, or individual responsible for publication charges.

MANUSCRIPT PREPARATION: STYLE AND FORM

Preparing the Manuscript File

Manuscripts should be submitted in Microsoft Word 2003 and should be double-spaced with lines and pages numbered consecutively using Times New Roman font at 12 points. Files created in Office 2007 should be saved down to Office 2003 before submission for compatibility with our composition software. All special characters (e.g., Greek, math, symbols) should be inserted using the symbols palette available in this font. Complex math should be entered using MathType or another equation editor. Tables and figures should be placed in separate sections at the end of the manuscripts (not placed in the text). Failure to follow these instructions may result in immediate rejection of the manuscript.

Metric or English units (or both) are acceptable. Authors should use units appropriate for the intended audience. Energy content of feeds will be expressed as calories.

Headings

Major Headings. Major headings are centered, boldface, in all capital letters, and consist of SUMMARY, DESCRIPTION OF PROBLEM, MATERIALS AND METHODS, RESULTS AND DISCUSSION, CONCLUSIONS AND APPLICATIONS, and REFERENCES AND NOTES.

Major headings in review articles, field reports, and symposium articles may vary from those listed here, but should include SUMMARY, CONCLUSIONS AND APPLICATIONS, and REFERENCES AND NOTES.

First Subheadings. First subheadings are boldface and italic, on a separate line beginning at the left margin, and have the first letter of each important word capitalized. Text that follows a first subheading should be in a new paragraph.

Second Subheadings. Second subheadings begin the first line of a paragraph. They are indented, boldface, italic, and followed by a period. The first letter of each important word is capitalized. The text follows immediately after the final period of the subheading.

Title Page

• The title should be indicative of the content. It should capture the interest of all who might benefit from information in the manuscript. However, the length of the title should be kept to a minimum.

• Address and affiliation of authors should be included. Indicate to whom correspondence should be directed by means of a footnote, with the notation "Corresponding author: (e-mail address)"
at the bottom of the title page. • List 3 to 8 key words or phrases to identify the most important subjects covered by the paper.

• The running title should be 30 characters or less, including spaces.

• Statement of primary audience: To determine appropriateness for the journal and to assist in selecting reviewers, the author should indicate clearly what sector(s) within the poultry community (e.g., flock supervisors, nutritionists, quality assurance personnel, researchers, plant managers, veterinarians) could most benefit from the content of this article.

Summary

The Summary (12 to 16 lines) is not an abstract. It is intended to give readers with diverse backgrounds a general appreciation of the manuscript contents. It should be written so that even those not directly interested in the topic will enjoy reading at least this section to keep abreast of areas other than their own. This section should not include details of materials and methods or a detailed review of the results. Keep the summary free-flowing, giving the reader a general, not specific, idea of what the study revealed. Do not include reference citations in the summary.

Description of Problem

This section will acquaint the reader with the problem, citing field experiences where appropriate. Readability is of utmost importance. Detailed literature reviews may not be appropriate for this section.

A more extensive citation of references should be included in the Results and Discussion or References and Notes section. This section should end with a statement of the objective(s) of the study.

Materials and Methods

The author(s) should clearly establish in the Materials and Methods section why the problem was approached in a particular way. The rationale for including each treatment should be clearly stated. Detailed laboratory and bird management procedures should be described in the References and Notes section and not in the Materials and Methods section. Sources of stock, equipment, and materials should be listed in the References and Notes section and not in the text or a footnote.

A brief statement of the statistical methods should be included, with more detailed descriptions placed in the References and Notes section.

In manuscripts using several treatments, a description of treatments should be included as Table 1.

Results and Discussion

This section begins with observed results and their interpretation. Descriptive subheadings may precede all major paragraphs and changes in subject emphasis. This section should discuss specifically how findings address the problem described in the Description of Problem section and how they are related to published works.

Statements regarding statistically significant differences between treatments in results should be included in the text, tables, and figures. Statements regarding differences should be avoided unless they are supported by statistical analyses and meet the stated level of probability (e.g., $P < 0.05$).

Conclusions and Applications

Conclusions and recommendations of the author(s) should be listed numerically. Each statement should be clear, concise, and without discussion. Authors are encouraged to summarize their significant findings, to identify further research needs, and to describe the constraints, economics, andother factors associated with using the results in scientific or commercial applications. Do not include references in this section.

References and Notes (with Acknowledgments)

References and notes should be cited in text, by number within an editorial bracket (e.g., [1]). In the References and Notes section, citations should be listed in the order they appear and are numbered in the text (not alphabetically). Authors are encouraged to use reference management software (e.g., EndNote or Reference Manager) to facilitate renumbering or inserting references by the editor or inserting references during the revision process. Manuscripts may be returned to authors *before review* for renumbering of references if not cited in numerical order. Include details such as statistical analysis; detailed procedures; sources of birds, instruments, or items; details of designed instruments; a literature review; and other tangential matters.

Cite acknowledgments at the end of this section in a subsection called *Acknowledgments*. These entries are not numbered.

Tables

Number tables consecutively according to the citation in the text. Tables must be created using the MS Word table feature and inserted in the manuscript after the references section. Each table must be placed on a separate page and must have a clear descriptive heading so that the meaning of the data will be understandable without reference to the text. Indicate footnotes to tables with numbers, beginning with 1. Statistical notation should be made with lowercase and uppercase superscript letters or with asterisks, as appropriate. Statistical notation should place the superscript “a” on the largest mean. Probability values may be indicated as follows: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, and $^{\dagger}P \leq 0.10$. Consult a recent issue of the journal for examples of tables.

Figures

• ***Figure Size***. Prepare figures at the final size for publication. Figures should be created at the final publication size of 8.9 cm wide (1 column), 14 cm wide (2 column), or 19 cm wide (full page width).

• ***Font Size***. Ensure that all type within the figure and axis labels is readable at the final publication size. A minimum type size of 8 points (after reduction) should be used.

• ***Fonts***. Use Helvetica or Times New Roman. Symbols may be inserted using

the Symbol palette in Times New Roman.
• ***Line Weight***. For line graphs, use a minimum stroke weight of 1 point for all lines. If multiple lines are to be distinguished, use solid, long dash, short dash, and dotted lines. Avoid the use of color, gray, or shaded lines because these will not reproduce well. Lines with different symbols for the data points may also be used to distinguish curves.
• ***Axis Labels***. Each axis should have a description and a unit. Units may be separated from the descriptor by a comma or parentheses, and should be consistent within a manuscript.
• ***Shading and Fill Patterns***. For bar charts, use different fill patterns if needed (e.g., black, white, gray, diagonal stripes). Avoid the use of multiple shades of gray because they will not be easily distinguishable in print.
• ***Symbols***. Identify curves and data points using the following symbols only: □, ■, ○, ●, ▲, ▼, △, ▽, ◊, ♦, +, or ×. Symbols should be defined in a key on the figure if possible.
• ***File Formats***. Figures can be submitted in Word, PDF, EPS, TIFF, and JPEG. Avoid Power-Point files and other formats. For the best printed quality, line art should be prepared at 600 ppi.
Grayscale and color images and photomicrographs should be at least 300 ppi.
• ***Grayscale Figures***. If figures are to be reproduced in grayscale (black and white), submit in grayscale. Often color will mask contrast problems that are apparent only when the figure is reproduced in grayscale.
• ***Color Figures***. If figures are to appear in color in the print journal, files must be submitted in CMYK color (not RGB).
• ***Photomicrographs***. Photomicrographs must have their unmagnified size designated, either in the caption or with a scale bar on the figure. Reduction for publication can make a magnification power designation (e.g., 100×) inappropriate.
• ***Caption***. The caption should provide sufficient information so that the figure can be understood without excessive reference to the text. All author-derived abbreviations used in the figure should be defined in the caption.
• ***General Tips***. Avoid the use of 3-dimensional bar charts unless essential to the presentation of the data. Use the simplest shading scheme possible to present the data clearly. Ensure that data, symbols, axis labels, lines, and the key are clear and easily readable at the final publication size.
Color Images. The cost to publish in color is $995 per figure; there is also a surcharge for color offprints. The corresponding author should complete a Color Charge Agreement form, available on the journal Web site (http://japr.fass.org/misc/ifora.dtl) and should fax (217-378-4083) that form to the JAPR editorial office when a manuscript with color figures is accepted for publication.

Sample References

NOTE: The headings that appear above the following sample references and notes are for clarification in these instructions, but they are not used in an actual paper, except for *Acknowledgments*.

Journal Article

Dansky, L. M., and F. W. Hill. 1952. Application of the chromic oxide indicator method to balance studies with growing chicks. J. Nutr. 47:449–459.

Snow, J. L., M. W. Douglas, and C. M. Parsons. 2003. Phytase effects on amino acid digestibility in molted laying hens. Poult. Sci. 82:474–477.

Witter, R. L., and I. M. Gimeno. 2006. Susceptibility of adult chickens, with and without prior vaccination, to challenge with Marek's disease virus. Avian Dis. doi:10.1637/7498-010306.R1

Monograph

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Dissertation

Heskett, E. A. 2003. Efficacy of a recombinant herpes virus of turkeys vector vaccine, expressing genes to Newcastle disease virus and Marek's disease virus, in chickens and turkeys against exotic Newcastle disease virus challenge. PhD Diss. Univ. Florida, Gainesville.

Trade Publication

Wilgus, H. S. 1973. Temperature-programmed feeding schedules and other means of conserving protein in market turkey production. Feedstuffs 45(27):27–31.

Book or Chapter in Book

AOAC International. 2007. Official Methods of Analysis of AOAC International. 18th ed. Rev. 2. AOAC Int., Gaithersburg, MD.

Whittow, G. C. 1976. Regulation of body temperature. Pages 146–173 in Avian Physiology. P. D. Sturkie, ed. Springer-Verlag, New York, NY.

Proceedings

Hruby, M., J. C. Remus, and E. E. M. Pierson. 2004. Nutritional strategies to meet the challenge of feeding poultry without antibiotic growth promotants. Pages 3–5 in Proc. 2nd Mid-Atlantic Nutr. Conf., Timonium, MD. Univ. Maryland, College Park.

Federal Register

USDA, Plant and Animal Health Inspection Service. 2004. Blood and tissue collections at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. Regist. 69:10137–10151.

Laboratory Procedure

The extract was added to 30 mL of hexane, made to 100 mL with 10% aqueous Na2SO4.

Personal Communication

Wilson, H. R. 2005. Univ. Florida, Gainesville. Personal communication.

Proprietary Product

Incubator, Petersime, Zulte, Belgium. Avizyme TX, Finnfeed International, Marlborough, Wiltshire, UK. Thymol, 99% purity, Acros Organics, Geel, Belgium.

Statistical Procedure

If a note has an embedded reference, the reference is cited by number (as in the text) or parenthetically within the note:

Data were analyzed by ANOVA with flock as the independent variable. When differences among flocks were significant, means were separated using Duncan's multiple range test (SAS User's Guide, 2001, Version 8 ed., SAS Institute Inc., Cary, NC). Pearson product-moment correlation coefficients were calculated between average percentage cracks from each flock recorded every week and average values for egg-specific gravity, breaking strength, percentage shell, shell thickness, and shell weight per unit of surface area. Significance implies *P* < 0.05.

Statistical Software

SAS User's Guide. 2001. Version 8 ed. SAS Inst. Inc., Cary, NC.

US Patent

El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, assignee. US Pat. No. 6,766,767.

Web Site

Dyro, F. M. 2005. Arsenic. WebMD. http://www.emedicine.com/neuro/topic20.htm Accessed Feb. 2006.

Acknowledgments

The advice and technical assistance of Thomas Jones (affiliation, location) are acknowledged.

Abbreviations

The following abbreviations may be used without definition in the *Journal of Applied Poultry Research*. Plurals do not require "s". Chemical symbols and 3-letter abbreviations for amino acids do not need definition. Other abbreviations should be defined at first use in the summary and the main text, as well as in each table or figure in which they appear. Abbreviations are boldface at first use in the main text. Abbreviations should not be used in the manuscript title, running title, or to begin a paragraph or sentence. They can be used in section headings if previously defined. This list appears inside the back cover of each issue of the journal.

ADF	acid detergent fiber
ADFI	average daily feed intake
ADG	average daily gain
AME	apparent metabolizable energy
AMEn	nitrogen-corrected apparent metabolizable energy
ANOVA	analysis of variance
BSA	bovine serum albumin
BW	body weight
℃	Celsius
cDNA	complementary DNA
CF	crude fiber
cfu	colony-forming units (following a numeral)
CI	confidence interval
CP	crude protein
cpm	counts per minute
CV	coefficient of variation
d	day
df	degrees of freedom
DM	dry matter
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetate
EE	ether extract
℉	Fahrenheit
FCR	feed conversion ratio
FE	feed efficiency

ft	foot
g	gram
gal	gallon
G:F	gain-to-feed ratio
GLM	general linear model
h	hour
HEPES	*N*-(2-hydroxyethyl)piperazine-*N′*-2-ethanesulfonic acid
HPLC	high-performance (high-pressure) liquid chromatography
ICU	international chick units
Ig	immunoglobulin
IL	interleukin
i.m.	intramuscular
in.	inch
i.p.	intraperitoneal
IU	international units
i.v.	intravenous
kcal	kilocalorie
L	liter (also capitalized with any combination, e.g., mL)
lb	pound
L:D	hours of light:hours of darkness in a photoperiod
LSD	least significant difference
m	meter
µ	micro
M	molar
ME	metabolizable energy
MEn	nitrogen-corrected metabolizable energy
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid
min	minute
mo	month
MS	mean squares
n	number of observations
N	normal
NAD	nicotinamide adenine dinucleotide
NADH	reduced form of NAD
NDF	neutral detergent fiber
NRC	National Research Council
NS	not significant
PBS	phosphate-buffered saline
ppm	parts per million
r	correlation coefficient
r2	coefficient of determination, simple
R2	coefficient of determination, multiple
RH	relative humidity
RIA	radioimmunoassay
RNA	ribonucleic acid
rpm	revolutions per minute
s	second
s.c.	subcutaneous

SD	standard deviation
SE	standard error
SEM	standard error of the mean
SNP	single nucleotide polymorphism
SRBC	sheep red blood cells
TBA	thiobarbituric acid
T	cell thymic-derived cell
TME	true metabolizable energy
TMEn	nitrogen-corrected true metabolizable energy
TSAA	total sulfur amino acids
USDA	United States Department of Agriculture
UV	ultraviolet
vol/vol	volume to volume
vs.	versus
wt/vol	weight to volume
wt/wt	weight to weight
wk	week
yr	year

Supplemental Information (Online)

The following information is available online and is updated regularly. Please refer to these pages when preparing a manuscript for submission.

Journal Title Abbreviations. A list of standard abbreviations for common journal titles is available online (http://japr.fass.org/misc/ifora.dtl).

SI Units. The following site (National Institute of Standards and Technology) provides a comprehensive guide to SI units and usage: http://physics.nist.gov/Pubs/SP811/contents.html

Manuscript Central Instructions. Manuscripts are submitted online (http://mc.manuscriptcentral. com/psa). Full user instructions for using the Manuscript Central system are available online; click the “Get Help Now” link on the top right of the main page (http://mc.manuscriptcentral.com/psa).

Apêndice 2. Normas para publicação de artigos no periódico Poultry Science

POULTRY SCIENCE INSTRUCTIONS TO AUTHORS

Editorial Policies and Procedures

Poultry Science publishes the results of fundamental and applied research concerning poultry, poultry products, and avian species in general. Submitted manuscripts shall provide new facts or confirmatory data. Papers dealing with experimental design, teaching, extension endeavors, or those of historical or biographical interest may also be appropriate. A limited number of review papers will be considered for publication if they contribute significant additional knowledge, or synthesis of knowledge, to a subject area. Papers that have been, or are scheduled to be, published elsewhere will not be accepted. Publication of a preliminary report, such as an abstract, does not preclude consideration of a complete report for publication as long as it has not been published in full in a proceedings or similar scientific publication; appropriate identification of previously published preliminary reports should be provided in a title page footnote. Translation of an article into other languages for publication requires approval by the editor-in-chief. Opinions or views expressed in papers published by *Poultry Science* are those of the author(s) and
do not necessarily represent the opinion of the Poultry Science Association or the editor-in-chief.

Contact Information for Journal Staff

For information on the scientific content of the journal, contact the editor-in-chief, Dr. Colin G. Scanes, 335 Chapman Hall, 2310 East Hartford Ave., University of Wisconsin, Milwaukee, WI 53201; e-mail: scanes@uwm.edu (with cc to cscanes@wi.rr.com). For assistance with Manuscript Central, manuscript submission and copyright forms, or page charge and offprint orders, contact Jeremy Holzner, editorial assistant, Headquarters Office, 2441 Village Green Place, Champaign, IL 61822 (FAX: 217-378-4083; jeremyh@assochq.org). For other information or to submit a paper, contact Susan Pollock, managing editor, Headquarters Office, Poultry Science Association, Inc., 2441 Village Green Place, Champaign, IL 61822 (telephone: 217-356-7641; FAX: 217- 378-4083; journals@assochq.org).

Care and Use of Animals

Authors must make it clear that experiments were conducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and laboratory techniques. Experiments shall be conducted in accordance with the principles and specific guidelines presented in *Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*, 1st revised edition, 1999 (Association Headquarters, 2441 Village Green Place, Champaign, IL 61822); and, if applicable,
Guide for the Care and Use of Laboratory Animals (United States Department of Human Health and Services, National Institutes of Health, Publication Number ISBN 0-309-05377-3, 1996); or *Guide to the Care and Use of ExperimentalAnimals,* 2nd ed. Volume 1, 1993 (Canadian Council on Animal Care). Methods of killing experimental animals must be described in the text. In

describing surgical procedures, the type and dosage of the anesthetic agent must be specified. Intra-abdominal and intrathoracic invasive surgery requires anesthesia. This includes caponization. The editor-in-chief of *Poultry Science* may refuse to publish manuscripts that are not compatible with these guides. If rejected solely on that basis, however, the paper may be resubmitted for reconsideration when accompanied by a written verification that a committee on animal care in research has approved the experimental design and procedures involved.

Types of Articles

Full-Length Articles. The majority of papers published in *Poultry Science* are full-length articles. The journal emphasizes the importance of good scientific writing and clarity in presentation of the concepts, apparatus, and sufficient background information that would be required for thorough understanding by scientists in other disciplines. The results of experiments published in *Poultry Science* must be replicated, either by replicating treatments within experiments or by repeating experiments.

Research Notes. Research Notes are short notes giving the results of complete experiments but are less comprehensive than full-length articles. Preliminary or progress reports will not be accepted. The running head shall be “RESEARCH NOTE.” Authors must also indicate the section under which the manuscript is to be reviewed on the title page of the manuscript and on the Manuscript Submission and Copyright Release Form. Research Notes will be published as a subsection of the scientific section in which they were reviewed. Research Notes are limited to five printed pages including tables and figures. Manuscripts should be prepared according to the guidelines for full-length articles.

Symposium Papers. The symposium organizer or chair must present the proposal and tentative budget to the Board of Directors at the summer meeting one full year before the symposium is to be scheduled. The symposium chair must then develop detailed symposium plans, including a formal outline of the talks approved and full budgetary expectations, which must be brought to the Board of Directors at the January meeting prior to the meeting at which the symposium is scheduled.

The symposium chair must decide whether or not the symposium is to be published and will inform the editor-in-chief of this decision at the January meeting. If the decision is not to publish the symposium, the individual authors retain the right to submit their papers for consideration for the journal as ordinary manuscripts. If publication is decided upon, all manuscript style and form guidelines of the journal shall be followed. Manuscripts must be prepared electronically, including figures and tables, and then uploaded onto the *Poultry Science* Manuscript Central site within 2 weeks after the annual meeting. The symposium chair will review the papers and, if necessary, return them to the authors for revision. The symposium chair then forwards the revised manuscript to the editor-in-chief for final review. Final revisions by the author and recommendations for acceptance or rejection by the chair must be completed by December 31 of the year in which the symposium was presented. Manuscripts not meeting this deadline will not be included in the published symposium proceedings. Symposium papers must be prepared in accordance with the guidelines for full-length articles and are subject to review. Offprints and costs of pages are the responsibility of the author.

Invited Papers. Invited papers, such as the World's Poultry Science Association lecture, should be submitted online; the editorial office will then make these papers available to the editor-in-chief. These papers are subject to review, and all manuscript style and form guidelines of the journal shall be followed. Invited papers are exempt from page charges but not offprint charges.

Review Papers. Review papers are accepted only if they provide new knowledge or a high-caliber synthesis of important knowledge. Reviews are not exempt from pages charges. All *Poultry Science* guidelines for style and form apply.

Invited Reviews. Invited Reviews will be approximately 10 published pages and in review format. The editor-in-chief will send invitations to the authors and then review these contributions when they are submitted. Nominations or suggestions for potential timely reviews are welcomed and should be sent directly to the editorin-chief.

Contemporary Issues. Contemporary Issues in *Poultry Science* will address critical issues facing poultry scientists and the poultry industry. As such, submissions to this section should be of interest to any poultry scientist, to the industry, to instructors and faculty teaching contemporary issues classes, and to undergraduate and graduate students. The section will consist of short papers (approximately 2 published pages) written in essay format and will include an abstract, appropriate subheadings, and references.

Rapid Communications. We aim for receipt-to-decision times of a month or less, and accepted papers will have priority for publication in the next available issue of *Poultry Science.* These papers will present informative and significant new findings, such as tissue-specific gene expression profile data with full-length cDNA and genomic gene structure characterization. These papers will be short (2 to 4 published pages), adhere to journal format, and include references and an abstract. Rapid Communications should **not** be preliminary reports or incomplete studies. Authors will select Rapid Communications as the paper type when submitting the paper.

Book Reviews. *Poultry Science* publishes reviews of books considered to be of interest to the readers. The editor-in-chief ordinarily solicits reviews. Unsolicited reviews must be sent directly to the editor-in-chief for approval. Book reviews shall be prepared in accordance to the style and form requirements of the journal, and they are subject to editorial revision. No page charges will be assessed.

Letters to the Editor. The purpose of letters will be to discuss, critique, or expand on scientific points made in articles recently published in *Poultry Science*. Introduction of unpublished data will not be allowed, nor will material based on conjecture or speculation. Letters must be received within 6 months of an article's publication. Letters will be limited to 400 words and 5 references (approximately 3 double-spaced, typed pages including references). Letters shall have a title. Author name(s) and affiliation(s) shall be placed between the end of the text and list of references. Letters will be sent electronically directly to the editor-in-chief for consideration. The author(s) of the original paper(s) will be provided a copy of the letter and offered the opportunity to submit for consideration a reply within 30 days. Replies will have the same page restrictions and format as letters, and the titles shall end with "—Reply." Letters and replies will be published together. Acceptability of letters will be decided by the editor-in-chief. Letters and replies shall follow appropriate *Poultry Science* format and may be edited by the editor-in-chief and a technical editor. If multiple letters on the same topic are received, a representative letter concerning a specific article will be published. All letters may not be published. Letters and replies will be published as space permits.

SUBMISSION OF ELECTRONIC MANUSCRIPTS

Authors should submit their papers electronically (http://mc.manuscriptcentral.com/ps). Detailed instructions for submitting electronically are provided online at that site. Authors who are unable to submit electronically should contact the editorial office (jeremyh@assochq.org) for assistance.

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Authors shall complete the Manuscript Submission and Copyright Release form for each new manuscript submission; faxed copies are acceptable. The form is published in *Poultry Science* as space permits and is available online (http://ps.fass.org). The copyright agreement is included in the Manuscript Submission and Copyright Release Form and must be completed by all authors before publication can proceed. The corresponding author is responsible for obtaining the signatures of coauthors. Persons unable to sign copyright agreements, such as federal employees, must indicate the reason for exemption on the form.

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REVIEW OF MANUSCRIPTS

After a manuscript is submitted electronically, the editorial office informs the appropriate section editor, who assigns two reviewers, at least one of whom is an associate editor. Each reviewer has 3 weeks to review the manuscript, after which his or her comments are forwarded to the section editor. The section editor may recommend rejection or acceptance at this point, after which the manuscript and reviewer comments are made available to the editor-in-chief for a final decision. More commonly, the manuscript will be sent back to the corresponding author for revision according to the guidelines of the reviewers. Authors have 6 weeks to complete the revision, which shall be returned to the section editor. Failure to return the manuscript within 6 weeks will cause the paper to be purged from the files. Purged manuscripts may be reconsidered, but they will have to be processed as new manuscripts.

Section editors handle all initial correspondence with authors during the review process. The editor-inchief will notify the author of the final decision to accept or reject. Rejected manuscripts can be resubmitted only with an invitation from the section editor or editor-inchief. Revised versions of previously rejected manuscripts are treated as new submissions. Therefore, authors must complete a new Manuscript Submission and Copyright Release Form.

PRODUCTION OF PROOFS

Accepted manuscripts are forwarded by the editor-inchief to the editorial office for technical editing and typesetting. At this point the technical editor may contact the authors for missing information or figure revisions. The manuscript is then typeset, figures reproduced, and author proofs prepared.

Proofs

Author proofs of all manuscripts will be provided to the corresponding author. Author proofs should be read carefully and checked against the typed manuscript, because the responsibility for proofreading is with the author(s). Corrections may be returned by fax, mail, or e-mail. For faxed or mailed corrections, changes to the proof should be made neatly and clearly in the margins of the proof. If extensive editing is required, corrections should be provided on a separate sheet of paper with a symbol indicating location on the proof. Changes sent by e-mail to the technical editor must indicate page, column, and line numbers for each correction to be made on the proof. Corrections can also be marked using the note and highlight tools to indicate necessary changes. Author alterations to copy exceeding 10% of the cost of composition will be charged to the author. Editor queries should be answered on the galley proofs; failure to do so may delay publication. Proof corrections should be made and returned to the technical editor within 48 hours of receipt.

Publication Charges and Offprints

Poultry Science has two options available for the publication of articles: conventional page charges and Open Access (**OA**).

OA. For authors who wish to publish their papers OA (freely available to everyone when the issue is posted online), authors will pay the OA fee when proofs are returned to the editorial office. Charges for OA are $2,400 if at least one author is a current professional member of PSA; the charge is $3,100 when no author is a professional member of PSA.

Conventional Page Charges. The current charge for publication is $100 per printed page (or fraction thereof) in the journal if at least one author is a professional member of PSA. If no author is a member of PSA, the publication charge is $170 per journal page.

Offprints and Color Charges. Offprints may be ordered at an additional charge. Authors who submit articles containing color illustrations are responsible for paying the additional charge for color printing, including the printing of any reprints they order, and must agree in writing prior to publication to pay the additional charges. When the galley proof is sent, the author is asked to complete an offprint order requesting the number of offprints desired and the name of the institution, agency, or individual responsible for publication charges.

MANUSCRIPT PREPARATION: STYLE AND FORM

General

Papers must be written in English. The text and all supporting materials must use American spelling and usage as given in *The American Heritage Dictionary, Webster's Third International Dictionary,* or the *Oxford American English Dictionary.* Authors should follow the style and form recommended in *Scientific Style and Format. The CBE Manual for Authors, Editors, and Publishers.* 6th ed. Council of Biology Editors Style Manual Committee. Cambridge Univ. Press, Cambridge, UK. Authors should prepare the main text, tables, and figure captions in MS Word. Details on figure preparation and file formats are provided in the Figures section of these instructions.

Preparing the Manuscript File

Manuscripts should be typed double-spaced, with lines and pages numbered consecutively, using Times New Roman font at 12 points. All special characters (e.g., Greek, math, symbols) should be inserted using the symbols palette available in this font. Complex math should be entered using MathType from Design Science (http://www.dessci.com). Equations created using the new Equation Builder feature in Microsoft Word 2007 may not be compatible with earlier versions of Word or other software used in our journal composition system. Tables and figures should be placed in separate sections at the end of the manuscript (not placed in the text). Failure to follow these instructions may result in an immediate rejection of the manuscript.

Headings

Major Headings. Major headings are centered (except ABSTRACT), all capitals, boldface, and consist of ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION (or RESULTS AND DISCUSSION), ACKNOWLEDGMENTS (optional), APPENDIX (optional), and REFERENCES.

First Subheadings. First subheadings are placed on a separate line, begin at the left margin, the first letter of all important words is capitalized, and the headings are boldface and italic. Text that follows a first subheading should be in a new paragraph.

Second Subheadings. Second subheadings begin the first line of a paragraph. They are indented, boldface, italic, and followed by a period. The first letter of each important word should be capitalized. The text follows immediately after the final period of the subheading.

Title Page

The title page shall begin with a running head (short title) of not more than 45 characters. The running head is centered, is in all capital letters, and shall appear on the top of the title page. No abbreviations should be used. The title of the paper must be in boldface; the first letter of the article title and proper names are capitalized, and the remainder of the title is lowercase. The title must have no abbreviations, and numbers must be given in words rather than in numerals (e.g., One-Day-Old Broilers).

Under the title, names of authors should be typed with initial capital letters and a space between initials

(e.g., T. E. Smith). Affiliations will be footnoted using the following symbols: *, †, ‡, §, #, ǁ, and be placed below the author names. Do not give authors' titles, positions, or degrees. Numbered footnotes may be used to provide supplementary information, such as present address, acknowledgment of grants, and experiment station or journal series number. The corresponding author should be indicated with a numbered footnote (e.g.,1Corresponding author: myname@university.edu). Note that there is no period after the corresponding author's e-mail address. The title page shall include the name and full address of the corresponding author. Telephone and FAX numbers and e-mail address must also be provided. The title page must indicate the appropriate scientific section for the paper (i.e., Education and Production; Environment, Well-Being, and Behavior; Genetics; Immunology, Health, and Disease; Metabolism and Nutrition; Molecular, Cellular, and Developmental Biology; Physiology, Endocrinology, and Reproduction; or Processing, Products, and Food Safety). Authors may create a full title page as a one-page document, in a file separate from the rest of the paper. This file can be uploaded and marked "not for review." Authors who choose to upload manuscripts with a full title page at the beginning will have their papers forwarded to reviewers as is.

Abbreviations

Author-derived abbreviations should be defined at first use in the abstract and again in the body of the manuscript. The abbreviation will be shown in bold type at first use in the body of the manuscript. Refer to the Miscellaneous Usage Notes for more information on abbreviations.

Abstract

The Abstract disseminates scientific information through abstracting journals and through convenience for the readers. The Abstract, consisting of not more than 325 words, appears at the beginning of the manuscript with the word ABSTRACT without a following period. It must summarize the major objectives, methods, results, conclusions, and practical applications of the research. The Abstract must consist of complete sentences and use of abbreviations should be limited. References to other work and footnotes are not permitted. The Abstract and Key Words must be on a separate sheet of paper.

Key Words

The Abstract shall be followed by a maximum of five key words or phrases to be used for subject indexing. These should include important words from the title and the running head and should be singular, not plural, terms (e.g., broiler, not broilers). Authors should consult a current "Subject Index " in *Poultry Science* for additional key words. Key words should be formatted as follows:

Key words: . . .

Introduction

The Introduction, while brief, should provide the reader with information necessary for understanding research presented in the paper. Previous work on the topic should be summarized, and the objectives of the current research must be clearly stated.

Materials and Methods

All sources of products, equipment, and chemicals used in the experiments must be specified parenthetically at first mention in text, tables, and figures [i.e., (model 123, ABC Corp., Provo, UT)]. Model and catalog numbers should be included. Information shall include the full corporate name (including division, branch, or other subordinate part of the corporation, if applicable), city, and state (country if outside the United States), or Web address. Street addresses need not be given unless the reader would not be able to determine the full address for mailing purposes easily by consulting standard references. Age, sex, breed, and strain or genetic stock of animals used in the experiments shall be specified. Animal care guidelines should be referenced if appropriate.

Papers must contain analyzed values for those dietary ingredients that are crucial to the experiment. In other papers, authors should state whether experimental diets meet or exceed the National Research Council (1994) requirements as appropriate. If not, crude protein and metabolizable energy levels should be stated. For layer diets, calcium and phosphorus contents should also be specified.

When describing the composition of diets and vitamin premixes, the concentration of vitamins A and E should be expressed as IU/kg on the basis of the following equivalents:

Vitamin A

1 IU = 0.3 μg of all-*trans* retinol

1 IU = 0.344 μg of retinyl acetate

1 IU = 0.552 μg of retinyl palmitate

1 IU = 0.60 μg of β-carotene

Vitamin E

1 IU = 1 mg of dl-α-tocopheryl acetate

1 IU = 0.91 mg of dl-α-tocopherol

1 IU = 0.67 mg of dl-α-tocopherol

In the instance of vitamin D3, cholecalciferol is the acceptable term on the basis that 1 IU of vitamin D3 = 0.025 μg of cholecalciferol.

The sources of vitamins A and E must be specified in parentheses immediately following the stated concentrations.

Statistical Analysis. Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statistical methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical analysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments separately. The experimental unit is the smallest unit to which an individual treatment is imposed. For group-fed animals, the group of animals in the pen is the experimental unit; therefore, groups must be replicated. Repeated chemical analyses of the same sample usually do not constitute independent experimental units. Measurements on the same experimental unit over time also are not independent and must not be considered as independent experimental units. For analysis of time effects, use timesequence analysis. Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to deviations from these assumptions, but occasionally data transformations or other techniques are helpful. For example, it is recommended that percentage data between 0 and 20 and between 80 and 100 be subjected to arc sin transformation prior to analysis. Most statistical procedures are based on the assumption that experimental units have been assigned to treatments at random. If animals are stratified by ancestry or weight or if some other initial measurement should be accounted for, the model should include a blocking factor, or the initial measurement should be included as a covariate. A parameter [mean (μ), variance (σ2)], which defines or describes a population, is estimated by a statistic (x, s2). The term **parameter** is not appropriate to describe a variable, observation, trait, characteristic, or measurement taken in an experiment.

Standard designs are adequately described by name and size (e.g., “a randomized complete block design with 6 treatments in 5 blocks”). For a factorial set of treatments, an adequate description might be as follows: “Total sulfur amino acids at 0.70 or 0.80% of the diet and Lys at 1.10%, 1.20%, or 1.30% of the diet were used in a 2 × 3 factorial arrangement in 5 randomized complete blocks consisting of initial BW.” Note that **a factorial arrangement is not a design**; the term “design” refers to the method of grouping experimental units into homogeneous groups or blocks (i.e., the way in which the randomization is restricted). Standard deviation refers to the variability in a sample or a population. The standard error (calculated from error variance) is the estimated sampling error of a statistic such as the sample mean. When a standard deviation or standard error is given, the number of degrees of freedom on which it rests should be specified. When any statistical value (as mean or difference of 2 means) is mentioned, its standard error or confidence limit should be given. The fact that differences are not “statistically significant” is no reason for omitting standard errors. They are of value when results from several experiments are combined in the future. They also are useful to the reader as measures of efficiency of experimental techniques. A value attached by “±” to a number implies that the second value is its standard error (not its standard deviation). Adequate reporting may require only 1) the number of observations, 2) arithmetic treatment means, and 3) an estimate of experimental error. The pooled standard error of the mean is the preferred estimate of experimental error. Standard errors need not be presented separately for each mean unless the means are based on different numbers of observations or the heterogeneity of the error variance is to be emphasized. Presenting individual standard errors clutters the presentation and can mislead readers.

For more complex experiments, tables of subclass means and tables of analyses of variance or covariance may be included. When the analysis of variance contains several error terms, such as in split-plot and repeated measures designs, the text should indicate clearly which mean square was used for the denominator of each *F* statistic. Unbalanced factorial data can present special problems.

Accordingly, it is well to state how the computing was done and how the parameters were estimated. Approximations should be accompanied by cautions concerning possible biases.

Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was designed; they should form the basis for comparing treatment means. Nonorthogonal contrasts may be evaluated by Bonferroni *t* statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally arranged. Fixed-range, pairwise, multiple-comparison tests should be used only to compare means of treatments that are unstructured or not related. Least squares means are the correct means to use for all data, but arithmetic means are identical to least squares means unless the design is unbalanced or contains missing values or an adjustment is being made for a covariate. In factorial treatment arrangements, means for main effects should be presented when important interactions are not present. However, means for individual treatment combinations also should be provided in table or text so that future researchers may combine data from several experiments to detect important interactions. An interaction may not be detected in a given experiment because of a limitation in the number of observations.

The terms significant and highly significant traditionally have been reserved for $P < 0.05$ and $P < 0.01$, respectively; however, reporting the *P*-value is preferred to the use of these terms. For example, use ". . . there was a difference ($P < 0.05$) between control and treated samples" rather than ". . . there was a significant ($P < 0.05$) difference between control and treated samples." When available, the observed significance level (e.g., $P = 0.027$) should be presented rather than merely $P < 0.05$ or $P < 0.01$, thereby allowing the reader to decide what to reject. Other probability (α) levels may be discussed if properly qualified so that the reader is not misled. Do not report *P*values to more than 3 places after the decimal. Regardless of the probability level used, failure to reject a hypothesis should be based on the relative consequences of type I and II errors. A "nonsignificant" relationship should not be interpreted to suggest the absence of a relationship.

An inadequate number of experimental units or insufficient control of variation limits the power to detect relationships. Avoid the ambiguous use of $P > 0.05$ to declare nonsignificance, such as indicating that a difference is not significant at $P > 0.05$ and subsequently declaring another difference significant (or a tendency) at $P < 0.09$. In addition, readers may incorrectly interpret the use of $P > 0.05$ as the probability of a β error, not an α error. Present only meaningful digits. A practical rule is to round values so that the change caused by rounding is less than one-tenth of the standard error. Such rounding increases the variance of the reported value by less than 1%, so that less than 1% of the relevant information contained in the data is sacrificed. In most cases, 2 or 3 significant digits (not decimal places) are sufficient.

Results and Discussion

Results and Discussion sections may be combined, or they may appear in separate sections. If separate, the Results section shall contain only the results and summary of the author's experiments; there should be no literature comparisons. Those comparisons should appear in the Discussion section.

Acknowledgments

An Acknowledgments section, if desired, shall follow the Discussion section. Acknowledgments of individuals should include affiliations but not titles, such as Dr., Mr., or Ms. Affiliations shall include institution, city, and state. Review copies shall have authors' institutions omitted.

Appendix

A technical Appendix, if desired, shall follow the Discussion section or Acknowledgments, if present. The Appendix may contain supplementary material, explanations, and elaborations that are not essential to othermajor sections but are helpful to the reader. Novel computer programs or mathematical computations would be appropriate. The Appendix will not be a repository for raw data.

References

Citations in Text. In the body of the manuscript, refer to authors as follows: Smith and Jones (1992) or Smith and Jones (1990, 1992). If the sentence structure requires that the authors' names be included in parentheses, the proper format is (Smith and Jones, 1982; Jones, 1988a,b; Jones et al., 1993). Where there are more than two authors of one article, the first author's name is followed by the abbreviation et al. More than one article listed in the same sentence of text must be in chronological order first, and alphabetical order for two publications in the same year. Work that has not been accepted for publication shall be listed in the text as: "J. E. Jones (institution, city, and state, personal communication)." The author's own unpublished work should be listed in the text as "(J. Smith, unpublished data)." Personal communications and unpublished data must not be included in the References section.

References Section. To be listed in the References section, papers must be published or accepted for publication. Manuscripts submitted for publication can be cited as "personal communication" or "unpublished data" in the text. Citation of abstracts, conference proceedings, and other works that have not been peer reviewed is strongly discouraged unless essential to the paper. Abstract and proceedings references are not apropriate citations in the Materials and Methods section of a paper. In the References section, references shall first be listed alphabetically by author(s)' last name(s), and then chronologically. The year of publication follows the authors' names. As with text citations, two or more publications by the same author or set of authors in the same year shall be differentiated by adding lowercase letters after the date. The dates for papers with the same first author that would be abbreviated in the text as et al., even though the second and subsequent authors differ, shall also be differentiated by letters. All authors' names must appear in the Reference section. Journals shall be abbreviated according to the conventional ISO abbreviations given in journals database of the National Library of Medicine (http://www.ncbi.nlm.nih.gov/entrez/query. fcgi?db=journals). One-word titles must be spelled out. Inclusive page numbers must be provided. Sample references are given below. Consult recent issues of *Poultry Science* for

examples not included below.
Article:
Bagley, L. G., and V. L. Christensen. 1991. Hatchability and physiology of turkey embryos incubated at sea level with increased eggshell permeability. Poult. Sci. 70:1412–1418. Bagley, L. G., V. L. Christensen, and R. P. Gildersleeve. 1990.
Hematological indices of turkey embryos incubated at high altitude as affected by oxygen and shell permeability. Poult. Sci. 69:2035–2039.
Witter, R. L., and I. M. Gimeno. 2006. Susceptibility of adult chickens, with and without prior vaccination, to challenge with Marek's disease virus. Avian Dis. doi:10.1637/7498- 010306R.1
Book:
Metcalfe, J., M. K. Stock, and R. L. Ingermann. 1984. The effects of oxygen on growth and development of the chick embryo. Pages 205-219 in Respiration and Metabolism of Embryonic Vertebrates. R. S. Seymour, ed. Dr. W. Junk, Dordrecht, the Netherlands.
National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.
Federal Register:
Department of Agriculture, Plant and Animal Health Inspection Service. 2004. Blood and tissue collection at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. Regist. 69:10137–10151.
Other:
Choct, M., and R. J. Hughes. 1996. Long-chain hydrocarbons as a marker for digestibility studies in poultry. Proc. Aust.Poult. Sci. Symp. 8:186. (Abstr.)
Dyro, F. M. 2005. Arsenic. WebMD. http://www.emedicine.com/ neuro/topic20.htm Accessed Feb. 2006.
El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, assignee. US Pat. No. 6,766,767.
Hruby, M., J. C. Remus, and E. E. M. Pierson. 2004. Nutritional strategies to meet the challenge of feeding poultry without antibiotic growth promotants. Proc. 2nd Mid-Atlantic Nutr. Conf., Timonium, MD. Univ. Maryland, College Park. Luzuriaga, D. A. 1999. Application of computer vision and electronic nose technologies for quality assessment of color and odor of shrimp and salmon. PhD Diss. Univ. Florida, Gainesville.
Peak, S. D., and J. Brake. 2000. The influence of feeding program on broiler breeder male mortality. Poult. Sci. 79(Suppl. 1):2. (Abstr.)

Tables

Tables must be created using the MS Word table feature and inserted in the manuscript after the references section. When possible, tables should be organized to fit across the page without running broadside. Be aware of the dimensions of the printed page when planning tables (use of more than 15 columns will create layout problems). Place the table number and title on the same line above the table. The table title does not require a period. Do not use vertical lines and use few horizontal lines. Use of bold and italic typefaces in the table body should be done sparingly; such use must be defined in a footnote. Each table must be on a separate page. To facilitate placement of all tables into the manuscript file (just after the references) authors should use "section breaks” rather than “page breaks” at the end of the manuscript (before the tables) and between tables. Units of measure for each variable must be indicated. Papers with several tables must use consistent format. All columns must have appropriate headings. Abbreviations not found on the inside front cover of the journal must be defined in each table and must match those used in the text. Footnotes to tables should be marked by superscript numbers. Each footnote should begin a new line. Superscript letters shall be used for the separation of means in the body of the table and explanatory footnotes must be provided [i.e., “Means within a row lacking a common superscript differ ($P < 0.05$).”]; other significant *P*-values may be specified. Comparison of means within rows and columns should be indicated by different series of superscripts (e.g., a,b, . . . in rows; x–z . . . in columns) The first alphabetical letter in the series (e.g., a or A) shall be used to indicate the largest mean. Lowercase superscripts indicate $P \leq 0.05$. Uppercase letters indicate $P \leq 0.01$ or less.

Probability values may be indicated as follows: *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and †$P \leq 0.10$. Consult a recent issue of *Poultry Science* for examples of tables.

Figures

To facilitate review, figures should be placed at the end of the manuscript (separated by section breaks). Each figure should be placed on a separate page, and identified by the manuscript number and the figure number. A figure with multiple panels or parts should appear on one page (e.g., if Figure 1 has parts a, b, and c, place all of these on the same page). Figure captions should be typed (double spaced) on a separate page.

• ***Figure Size.*** Prepare figures at final size for publication. Figures should be prepared to fit one column
(8.9 cm wide), 2 columns (14 cm wide), or full-page width (19 cm wide).

• ***Font Size.*** Ensure that all type within the figure and axis labels are readable at final publication size.
A minimum type size of 8 points (after reduction) should be used.

• ***Fonts.*** Use Helvetica or Times New Roman. Symbols may be inserted using the Symbol palette in Times New Roman.

• ***Line Weight.*** For line graphs, use a minimum stroke weight of 1 point for all lines. If multiple lines are to be distinguished, use solid, long-dash, short-dash, and dotted lines. Avoid the use of color, gray, or
shaded lines, as these will not reproduce well. Lines with different symbols for the data points may also be used to distinguish curves.

• ***Axis Labels.*** Each axis should have a description and a unit. Units may be separated from the descriptor by a comma or parentheses, and should be consistent within a manuscript.

• ***Shading and Fill Patterns.*** For bar charts, use different fill patterns if needed (e.g., black, white, gray, diagonal stripes). Avoid the use of multiple shades of gray, as they will not be easily distinguishable in print.

• ***Symbols.*** Identify curves and data points using the following symbols only: □, ■, ○, ●, ▲, ▼, n, ,, e, r, +, or ×. Symbols should be defined in a key on the figure if possible.

• ***File Formats.*** Figures can be submitted in Word, PDF, EPS, TIFF, and JPEG. Avoid PowerPoint files and other formats. For the best printed quality, line art should be prepared at 600 ppi. Grayscale and color images and

photomicrographs should be at least 300 ppi.
• ***Grayscale Figures.*** If figures are to be reproduced in grayscale (black and white), submit in grayscale. Often color will mask contrast problems that are apparent only when the figure is reproduced in grayscale.
• ***Color Figures.*** If figures are to appear in color in the print journal, files must be submitted in CMYK color (not RGB).
• ***Photomicrographs.*** Photomicrographs must have their unmagnified size designated, either in the caption or with a scale bar on the figure. Reduction for publication can make a magnification power designation (e.g., 100×) inappropriate.
• ***Caption.*** The caption should provide sufficient information that the figure can be understood with excessive reference to the text. All author-derived abbreviations used in the figure should be defined in the caption.
• ***General Tips.*** Avoid the use of three-dimensional bar charts, unless essential to the presentation of the data. Use the simplest shading scheme possible to present the data clearly. Ensure that data, symbols, axis labels, lines, and key are clear and easily readable at final publication size.

Color Figures. Submitted color images should be at least 300 ppi. The cost to publish each color figure is $995; a surcharge for color reprints ordered will be assessed. Authors must agree in writing to bear the costs of color production after acceptance and prior to publication of the paper. The form “Color Charge Agreement” is available on the journal web site (http://ps.fass.org) and should be completed and returned to PSA Headquarters upon submission.

Miscellaneous Usage Notes

Abbreviations. Abbreviations shall not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by abbreviation (e.g., IgG, CD).

A helpful criterion for use of abbreviation is whether it has been accepted into thesauri and indexes widely used for searching major bibliographic databases in the scientific field. Abbreviations may be used in heads within the paper, if they have been first defined within the text. The inside back cover of every issue of the journal lists ab-breviations that can be used without definition. The list is subject to revision at any time, so authors should always consult the most recent issue of the journal (or the updated list at http://ps.fass.org/) for relevant information. Abbreviations are allowed when they help the flow of the manuscript; however, excessive use of abbreviations can confuse the reader. The suitability of abbreviations will be evaluated by the reviewers and editors during the review process and by the technical editor during editing. As a rule, author-derived abbreviations should be in all capital letters. Terms used less than three times must be spelled out in full rather than abbreviated. All terms are to be spelled out in full with the abbreviation following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbreviations shall be used consistently thereafter, rather than the full term. The abstract, text, each table, and each figure must be understood independently of each other. Therefore, abbreviations shall be defined within each of these units of the manuscript.

Plural abbreviations do not require “s.” Chemical symbols and three-letter abbreviations for amino acids do not need definition. Units of measure, except those in the standard *Poultry Science* abbreviation list, should be abbreviated as listed in the *CRC Handbook for Chemistry and Physics* (CRC Press, 2000 Corporate Blvd., Boca Raton, FL 33431) and do not need to be defined. The following abbreviations may be used without definition in *Poultry Science.*

A	adenine
ADG	average daily gain
ADFI	average daily feed intake
AME	apparent metabolizable energy
AMEn	nitrogen-corrected apparent metabolizable energy
ANOVA	analysis of variance
B	cell bursal-derived, bursal-equivalent derived cell
bp	base pairs
BSA	bovine serum albumin
BW	body weight
C	cytosine
cDNA	complementary DNA
cfu	colony-forming units
CI	confidence interval
CP	crude protein
cpm	counts per minute
CV	coefficient of variation
d	day
df	degrees of freedom
DM	dry matter
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetate
ELISA	enzyme-linked immunosorbent antibody assay
EST	expressed sequence tag
g	gram
g	gravity
G	guanine
GAT	glutamic acid-alanine-tyrosine
G:F	gain-to-feed ratio
GLM	general linear model
h	hour
HEPES	*N*-2-hydroxyethyl piperazine-*N′*-ethane-sulfonic acid
HPLC	high-performance (high-pressure) liquid chromatography
ICU	international chick units
Ig	immunoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IU	international units
i.v.	intravenous
kb	kilobase pairs
kDa	kilodalton
L	liter*
L:D	hours light:hours darkness in a photoperiod
m	meter
µ	micro

M	molar
MAS	marker-assisted selection
ME	metabolizable energy
MEn	nitrogen-corrected metabolizable energy
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid
min	minute
mo	month
MS	mean square
n	number of observations
N	normal
NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NRC	National Research Council
NS	not significant
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
pfu	plaque-forming units
QTL	quantitative trait loci
r	correlation coefficient
r2	coefficient of determination, simple
R2	coefficient of determination, multiple
RFLP	restriction fragment length polymorphism
RH	relative humidity
RIA	radioimmunoassay
RNA	ribonucleic acid
rpm	revolutions per minute
s	second
s.c.	subcutaneous
SD	standard deviation
SDS	sodium dodecyl sulfate
SE	standard error
SEM	standard error of the mean
SRBC	sheep red blood cells
SNP	single nucleotide polymorphism
T	thymine
TBA	thiobarbituric acid
T	cell thymic-derived cell
TME	true metabolizable energy
TMEn	nitrogen-corrected true metabolizable energy
Tris	tris(hydroxymethyl)aminomethane
TSAA	total sulfur amino acids
U	uridine
USDA	United States Department of Agriculture
UV	ultraviolet
vol/vol	volume to volume
vs.	versus

wt/vol	weight to volume
wt/wt	weight to weight
wk	week
yr	year

*Also capitalized with any combination, e.g., mL.

International Words and Phrases. Non-English words in common usage (defined in recent editions of standard dictionaries) will not appear in italics (e.g., in vitro, in vivo, in situ, a priori). However, genus and species of plants, animals, or bacteria and viruses should be italicized. Authors must indicate accent marks and other diacriticals on international names and institutions. German nouns shall begin with capital letters.

Capitalization. Breed and variety names are to be capitalized (e.g., Single Comb White Leghorn).

Number Style. Numbers less than 1 shall be written with preceding zeros (e.g., 0.75). All numbers shall be written as digits. Measures must be in the metric system; however, US equivalents may be given in parentheses.

Poultry Science requires that measures of energy be given in calories rather than joules, but the equivalent in joules may be shown in parentheses or in a footnote to tables. Units of measure not preceded by numbers must be written out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically. Measures of variation must be defined in the Abstract and in the body of the paper at first use. Units of measure for feed conversion or feed efficiency shall be provided (i.e., g:g).

Nucleotide Sequences. Nucleotide sequence data must relate to poultry or poultry pathogens and must complement biological data published in the same or a companion paper. If sequences are excessively long, it is suggested that the most relevant sections of the data be published in *Poultry Science* and the remaining sequences be submitted to one of the sequence databases. Acceptance for publication is contingent on the submission of sequence data to one of the databases. The following statement should appear as a footnote to the title on the title page of the manuscript. “The nucleotide sequence data reported in this paper have been submitted to GenBank Submission (Mail Stop K710, Los Alamos National Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN.” Publication of the description of molecular clones is assumed by the editors to place them in the public sector.

Therefore, they shall be made available to other scientists for research purposes. Nucleotide sequences must be submitted as cameraready figures no larger than 21.6 × 27.9 cm in standard (portrait) orientation. Abbreviations should follow *Poultry Science* guidelines.

General Usage. Note that “and/or” is not permitted;choose the more appropriate meaning or use “x or y or both.”

Use the slant line only when it means “per” with numbered units of measure or “divided by” in equations. Use only one slant line in a given expression (e.g., g/d per chick). The slant line may not be used to indicate ratios or mixtures.

Use “to” instead of a hyphen to indicate a range. Insert spaces around all signs (except slant lines) of operation (=, –, +, ×, >, or <, etc.) when these signs occur between two items. Items in a series should be separated by commas (e.g., a, b, and c). Restrict the use of "while" and "since" to meanings related to time. Appropriate substitutes include "and," "but," or "whereas" for "while" and "because" or "although" for "since." Leading (initial) zeros should be used with numbers less than 1 (e.g., 0.01). Commas should be used in numbers greater than 999.

Registered (®) and trademark (™) symbols should not be used, unless as part of an article title in the References section. Trademarked product names should be capitalized.

Supplemental Information (Online)

The following information is available online and updated regularly. Please refer to these pages when preparing a manuscript for submission.

Journal Title Abbreviations. A list of standard abbreviations for common journal titles is available online (http://ps.fass.org/misc/ifora.dtl).

SI Units. The following site (National Institute of Standards and Technology) provides a comprehensive guide to SI units and usage: http://physics.nist.gov/Pubs/SP811/ contents.html

Figure and Table Preparation Guidelines. Current detailed information on figure and table preparation can be found at http://ps.fass.org/misc/ifora.dtl

Manuscript Central Instructions. Manuscripts are submitted online (http://mc.manuscriptcentral.com/psa). Full user instructions for using the Manuscript Central system are available on the Mansuscript Central home page.

Apêndice 3. Temperatura e umidade média durante o experimento

CONTROLE DIÁRIO DE TEMPERATURA							
DATA	**IDADE (SEM)**	**T ℃ Min**	**T ℃ Máx.**	**T ℃ Média**	**UR % Min**	**UR % Máx.**	**UR % Média**
21/03/2010	21	19,20	27,90	23,55	70	98	84,0
22/03/2010	21	19,50	28,90	24,20	60	99	79,5
23/03/2010	21	19,10	26,80	22,95	70	92	81,0
24/03/2010	21	20,50	24,80	22,65	70	91	80,5
25/03/2010	21	23,80	25,90	24,85	61	89	75,0
26/03/2010	21	22,00	25,00	23,50	70	87	78,5
27/03/2010	22	22,90	29,20	26,05	66	91	78,5
28/03/2010	22	23,40	28,80	26,10	61	85	73,0
29/03/2010	22	21,00	28,20	24,60	62	84	73,0
30/03/2010	22	19,00	25,30	22,15	73	87	80,0
31/03/2010	22	19,00	28,50	23,75	56	82	69,0
01/04/2010	22	21,20	27,90	24,55	49	82	65,5
02/04/2010	22	24,00	29,40	26,70	85	87	86,0
03/04/2010	23	21,40	30,80	26,10	47	84	65,5
04/04/2010	23	19,30	26,60	22,95	76	87	81,5
05/04/2010	23	17,50	20,50	19,00	67	81	74,0
06/04/2010	23	12,40	23,60	18,00	43	79	61,0
07/04/2010	23	14,60	21,90	18,25	42	79	60,5
08/04/2010	23	15,90	23,90	19,90	52	79	65,5
09/04/2010	23	18,60	23,50	21,05	49	77	63,0
10/04/2010	24	16,30	23,50	19,90	44	81	62,5
11/04/2010	24	17,30	25,90	21,60	46	75	60,5
12/04/2010	24	20,00	27,70	23,85	46	76	61,0
13/04/2010	24	18,20	27,60	22,90	63	78	70,5
14/04/2010	24	17,80	28,50	23,15	42	78	60,0
15/04/2010	24	19,50	28,40	23,95	48	82	65,0
16/04/2010	24	13,30	22,10	17,70	48	82	65,0
17/04/2010	25	12,00	24,20	18,10	46	72	59,0
18/04/2010	25	16,10	26,70	21,40	44	78	61,0
19/04/2010	25	19,80	31,00	25,40	47	76	61,5
20/04/2010	25	19,60	29,80	24,70	46	90	68,0
21/04/2010	25	19,30	23,20	21,25	83	95	89,0
22/04/2010	25	19,40	24,60	22,00	76	96	86,0
23/04/2010	25	16,10	23,70	19,90	76	96	86,0
24/04/2010	26	15,30	18,30	16,80	64	96	80,0
25/04/2010	26	18,00	24,10	21,05	71	85	78,0
26/04/2010	26	20,20	24,80	22,50	73	90	81,5
27/04/2010	26	18,50	23,50	21,00	82	97	89,5
28/04/2010	26	12,30	21,60	16,95	75	82	78,5
29/04/2010	26	12,60	21,10	16,85	46	75	60,5
30/04/2010	26	16,60	22,20	19,40	48	74	61,0
01/05/2010	27	14,60	21,20	17,90	48	78	63,0
02/05/2010	27	10,50	22,00	16,25	52	80	66,0
03/05/2010	27	13,90	24,50	19,20	46	73	59,5
04/05/2010	27	24,90	25,50	25,20	84	88	86,0
05/05/2010	27	17,20	24,00	20,60	60	84	72,0

06/05/2010	27	15,40	24,70	20,05	56	79	67,5
07/05/2010	27	16,50	19,50	18,00	61	80	70,5
08/05/2010	28	15,20	20,50	17,85	65	87	76,0
09/05/2010	28	15,50	19,90	17,70	46	75	60,5
10/05/2010	28	15,60	20,90	18,25	46	76	61,0
11/05/2010	28	17,10	19,20	18,15	64	83	73,5
12/05/2010	28	16,00	19,80	17,90	69	90	79,5
13/05/2010	28	17,00	27,20	22,10	64	82	73,0
14/05/2010	28	14,00	22,90	18,45	77	75	76,0
15/05/2010	29	15,30	22,00	18,65	56	81	68,5
16/05/2010	29	17,10	24,70	20,90	52	83	67,5
17/05/2010	29	14,00	24,70	19,35	61	83	72,0
18/05/2010	29	18,10	20,10	19,10	58	79	68,5
19/05/2010	29	16,20	19,90	18,05	78	84	81,0
20/05/2010	29	15,60	19,60	17,60	71	80	75,5
21/05/2010	29	17,30	20,70	19,00	83	88	85,5
22/05/2010	30	19,20	23,00	21,10	73	87	80,0
23/05/2010	30	19,10	23,50	21,30	65	85	75,0
24/05/2010	30	19,80	22,10	20,95	61	82	71,5
25/05/2010	30	18,80	18,90	18,85	61	86	73,5
26/05/2010	30	14,20	19,80	17,00	77	86	81,5
27/05/2010	30	18,90	20,70	19,80	57	82	69,5
28/05/2010	30	17,50	21,90	19,70	74	89	81,5
29/05/2010	31	17,70	17,90	17,80	60	88	74,0
30/05/2010	31	17,70	22,20	19,95	83	94	88,5
31/05/2010	31	12,20	23,00	17,60	61	86	73,5
01/06/2010	31	14,90	17,50	16,20	47	76	61,5
02/06/2010	31	12,70	18,30	15,50	75	79	77,0
03/06/2010	31	12,50	19,50	16,00	49	73	61,0
04/06/2010	31	12,60	22,10	17,35	53	78	65,5
05/06/2010	32	16,20	21,30	18,75	72	82	77,0
06/06/2010	32	12,70	18,70	15,70	44	82	63,0
07/06/2010	32	9,80	19,80	14,80	67	70	68,5
08/06/2010	32	13,10	19,80	16,45	54	76	65,0
09/06/2010	32	11,30	18,60	14,95	52	78	65,0
10/06/2010	32	9,30	15,90	12,60	48	72	60,0
11/06/2010	32	11,10	15,20	13,15	76	80	78,0
12/06/2010	33	14,10	19,40	16,75	49	68	58,5
13/06/2010	33	12,60	19,60	16,10	43	77	60,0
14/06/2010	33	12,90	19,90	16,40	57	75	66,0
15/06/2010	33	17,30	21,20	19,25	54	77	65,5
16/06/2010	33	18,70	23,70	21,20	54	95	74,5
17/06/2010	33	17,20	20,30	18,75	76	95	85,5
18/06/2010	33	16,30	18,90	17,60	83	86	84,5
19/06/2010	34	19,50	23,80	21,65	60	87	73,5
20/06/2010	34	13,90	22,60	18,25	80	86	83,0
21/06/2010	34	10,10	16,60	13,35	63	80	71,5
22/06/2010	34	8,30	17,30	12,80	69	78	73,5
23/06/2010	34	7,50	16,40	11,95	56	71	63,5
24/06/2010	34	9,50	15,20	12,35	45	76	60,5
25/06/2010	34	15,00	24,30	19,65	50	81	65,5

26/06/2010	35	14,90	17,70	16,30	78	89	83,5
27/06/2010	35	16,50	21,20	18,85	78	91	84,5
28/06/2010	35	15,30	25,70	20,50	60	83	71,5
29/06/2010	35	12,10	19,00	15,55	67	80	73,5
30/06/2010	35	17,30	20,30	18,80	76	83	79,5
01/07/2010	35	16,40	22,60	19,50	67	85	76,0
02/07/2010	35	15,90	18,80	17,35	69	85	77,0
03/07/2010	36	14,00	24,30	19,15	76	79	77,5
04/07/2010	36	14,30	23,60	18,95	57	79	68,0
05/07/2010	36	13,10	23,40	18,25	52	78	65,0
06/07/2010	36	17,40	24,70	21,05	73	87	80,0
07/07/2010	36	15,70	26,00	20,85	48	72	60,0
08/07/2010	36	14,80	27,50	21,15	42	75	58,5
09/07/2010	36	10,10	19,90	15,00	75	82	78,5
10/07/2010	37	12,70	19,20	15,95	73	76	74,5
11/07/2010	37	14,40	18,10	16,25	65	81	73,0
12/07/2010	37	16,40	24,10	20,25	57	84	70,5
13/07/2010	37	8,70	19,30	14,00	64	87	75,5
14/07/2010	37	7,50	12,20	9,85	38	72	55,0
15/07/2010	37	5,40	13,70	9,55	44	68	56,0
16/07/2010	37	7,60	15,20	11,40	41	87	64,0
17/07/2010	38	7,80	14,80	11,30	38	85	61,5
18/07/2010	38	14,20	17,90	16,05	75	83	79,0
19/07/2010	38	16,10	18,70	17,40	80	84	82,0
20/07/2010	38	13,20	18,50	15,85	58	82	70,0
21/07/2010	38	9,30	14,30	11,80	52	78	65,0
22/07/2010	38	9,30	21,60	15,45	52	84	68,0
23/07/2010	38	13,50	16,60	15,05	56	86	71,0
24/07/2010	39	8,90	16,40	12,65	54	75	64,5
25/07/2010	39	12,50	17,60	15,05	66	84	75,0
26/07/2010	39	12,00	21,10	16,55	73	83	78,0
27/07/2010	39	8,90	15,90	12,40	53	79	66,0
28/07/2010	39	8,70	17,80	13,25	69	79	74,0
29/07/2010	39	13,30	15,10	14,20	54	77	65,5
30/07/2010	39	14,40	24,20	19,30	47	79	63,0
31/07/2010	40	11,50	19,70	15,60	69	84	76,5
01/08/2010	40	7,20	17,70	12,45	55	75	65,0
02/08/2010	40	6,50	15,50	11,00	38	78	58,0
03/08/2010	40	5,50	14,90	10,20	45	74	59,5
04/08/2010	40	6,70	13,80	10,25	42	71	56,5
05/08/2010	40	6,50	14,40	10,45	50	75	62,5
06/08/2010	40	12,10	14,70	13,40	68	76	72,0
07/08/2010	41	11,70	17,30	14,50	58	74	66,0
08/08/2010	41	11,70	17,90	14,80	66	80	73,0
09/08/2010	41	10,50	17,60	14,05	75	86	80,5
10/08/2010	41	8,60	16,90	12,75	40	66	53,0
11/08/2010	41	9,20	17,00	13,10	31	77	54,0
12/08/2010	41	15,80	21,40	18,60	51	70	60,5
13/08/2010	41	12,10	18,80	15,45	68	73	70,5
14/08/2010	42	13,50	21,10	17,30	66	86	76,0
15/08/2010	42	13,20	16,40	14,80	58	79	68,5

16/08/2010	42	14,00	18,50	16,25	46	76	61,0
17/08/2010	42	13,70	19,50	16,60	47	81	64,0
18/08/2010	42	13,80	20,40	17,10	42	72	57,0
19/08/2010	42	11,70	22,20	16,95	48	69	58,5
20/08/2010	42	13,00	23,30	18,15	46	72	59,0
21/08/2010	43	11,20	20,00	15,60	36	70	53,0
22/08/2010	43	11,30	22,80	17,05	43	71	57,0
23/08/2010	43	13,70	27,20	20,45	49	75	62,0
24/08/2010	43	17,40	29,50	23,45	29	74	51,5
25/08/2010	43	17,00	25,90	21,45	49	76	62,5
26/08/2010	43	17,20	19,20	18,20	84	88	86,0
27/08/2010	43	17,10	20,20	18,65	80	87	83,5
28/08/2010	44	17,40	19,90	18,65	83	90	86,5
29/08/2010	44	18,80	21,70	20,25	82	85	83,5
30/08/2010	44	14,30	24,00	19,15	64	84	74,0
31/08/2010	44	14,10	24,20	19,15	74	86	80,0
01/09/2010	44	16,90	25,20	21,05	49	78	63,5
02/09/2010	44	18,30	23,70	21,00	87	91	89,0
03/09/2010	44	18,60	20,50	19,55	85	92	88,5
04/09/2010	45	13,40	22,10	17,75	84	99	91,5
05/09/2010	45	13,80	17,70	15,75	57	82	69,5
06/09/2010	45	11,90	20,70	16,30	44	72	58,0
07/09/2010	45	14,00	19,70	16,85	33	74	53,5
08/09/2010	45	15,50	20,50	18,00	48	77	62,5
09/09/2010	45	21,90	24,50	23,20	48	77	62,5
10/09/2010	45	14,10	22,10	18,10	39	72	55,5
11/09/2010	46	15,20	24,20	19,70	33	74	53,5
12/09/2010	46	15,20	24,70	19,95	57	88	72,5
13/09/2010	46	15,40	24,20	19,80	84	99	91,5
14/09/2010	46	17,10	19,60	18,35	69	95	82,0
15/09/2010	46	13,90	19,10	16,50	49	72	60,5
16/09/2010	46	11,30	19,80	15,55	40	76	58,0
17/09/2010	46	17,70	21,70	19,70	32	75	53,5
18/09/2010	47	10,80	21,50	16,15	65	78	71,5
19/09/2010	47	10,50	18,70	14,60	31	66	48,5
20/09/2010	47	13,70	22,40	18,05	32	67	49,5
21/09/2010	47	17,60	25,40	21,50	40	81	60,5
22/09/2010	47	17,90	20,50	19,20	72	84	78,0
23/09/2010	47	16,60	22,60	19,60	56	88	72,0
24/09/2010	47	16,50	20,20	18,35	72	85	78,5
25/09/2010	48	15,60	20,40	18,00	56	87	71,5
26/09/2010	48	16,80	20,60	18,70	57	74	65,5
27/09/2010	48	16,80	21,50	19,15	54	73	63,5
28/09/2010	48	16,50	23,50	20,00	53	74	63,5
29/09/2010	48	15,90	20,50	18,20	66	79	72,5
30/09/2010	48	14,00	24,30	19,15	36	78	57,0
01/10/2010	48	12,40	23,10	17,75	33	70	51,5
02/10/2010	49	8,60	23,20	15,90	37	67	52,0
03/10/2010	49	12,50	24,60	18,55	48	76	62,0
04/10/2010	49	16,10	19,40	17,75	33	67	50,0
05/10/2010	49	13,70	21,70	17,70	36	64	50,0

06/10/2010	49	13,40	24,50	18,95	35	70	52,5
07/10/2010	49	17,70	22,20	19,95	42	96	69,0
08/10/2010	49	16,50	22,30	19,40	66	76	71,0
09/10/2010	50	16,20	21,40	18,80	55	78	66,5
10/10/2010	50	14,60	21,20	17,90	39	78	58,5
11/10/2010	50	17,50	20,80	19,15	35	65	50,0
12/10/2010	50	11,70	20,90	16,30	30	68	49,0
13/10/2010	50	14,70	22,80	18,75	31	66	48,5
14/10/2010	50	17,50	24,40	20,95	30	64	47,0
15/10/2010	50	17,70	23,10	20,40	59	71	65,0
16/10/2010	51	17,60	26,70	22,15	44	83	63,5
17/10/2010	51	18,30	24,10	21,20	57	78	67,5
18/10/2010	51	14,40	21,30	17,85	54	67	60,5
19/10/2010	51	11,50	24,10	17,80	30	69	49,5
20/10/2010	51	14,70	24,00	19,35	31	66	48,5
21/10/2010	51	14,50	25,60	20,05	28	71	49,5
22/10/2010	51	16,30	26,70	21,50	30	72	51,0
23/10/2010	52	18,00	25,10	21,55	42	76	59,0
24/10/2010	52	15,70	25,90	20,80	49	83	66,0
25/10/2010	52	15,40	25,00	20,20	52	69	60,5
26/10/2010	52	14,40	25,40	19,90	49	64	56,5
27/10/2010	52	13,90	23,60	18,75	27	65	46,0
28/10/2010	52	14,90	23,30	19,10	34	62	48,0
29/10/2010	52	14,30	25,50	19,90	33	65	49,0
30/10/2010	53	14,00	24,30	19,15	40	71	55,5
31/10/2010	53	12,60	24,00	18,30	55	77	66,0
01/11/2010	53	16,50	26,30	21,40	20	61	40,5
02/11/2010	53	12,60	26,00	19,30	22	58	40,0
03/11/2010	53	12,70	27,50	20,10	19	59	39,0
04/11/2010	53	14,80	29,20	22,00	20	57	38,5
05/11/2010	53	19,50	33,10	26,30	17	70	43,5
06/11/2010	54	14,00	33,10	23,55	52	68	60,0
07/11/2010	54	13,60	33,10	23,35	20	68	44,0
08/11/2010	54	16,70	31,00	23,85	18	53	35,5
09/11/2010	54	16,80	34,30	25,55	20	67	43,5
10/11/2010	54	12,10	34,00	23,05	38	76	57,0
11/11/2010	54	11,30	32,00	21,65	26	56	41,0
12/11/2010	54	10,30	34,30	22,30	28	52	40,0
13/11/2010	55	10,90	23,40	17,15	30	63	46,5
14/11/2010	55	17,30	25,30	21,30	32	73	52,5
15/11/2010	55	17,00	26,50	21,75	37	68	52,5
16/11/2010	55	15,70	27,20	21,45	35	79	57,0
17/11/2010	55	15,00	29,20	22,10	28	71	49,5
18/11/2010	55	15,20	29,00	22,10	19	57	38,0
19/11/2010	55	15,00	29,20	22,10	20	70	45,0
20/11/2010	56	13,80	29,00	21,40	42	71	56,5
21/11/2010	56	17,70	28,10	22,90	23	73	48,0
22/11/2010	56	19,30	25,70	22,50	43	78	60,5
23/11/2010	56	18,60	27,10	22,85	56	86	71,0
24/11/2010	56	18,80	26,00	22,40	43	75	59,0
25/11/2010	56	18,20	25,30	21,75	75	86	80,5

26/11/2010	56	18,00	27,00	22,50	61	90	75,5
27/11/2010	57	15,70	23,80	19,75	39	83	61,0
28/11/2010	57	15,70	29,90	22,80	19	81	50,0
29/11/2010	57	20,00	28,80	24,40	38	65	51,5
30/11/2010	57	19,00	27,20	23,10	53	70	61,5
01/12/2010	57	13,90	28,80	21,35	21	65	43,0
02/12/2010	57	21,60	29,00	25,30	19	63	41,0
03/12/2010	57	13,90	22,40	18,15	21	73	47,0
04/12/2010	58	13,00	32,00	22,50	36	56	46,0
05/12/2010	58	13,90	32,10	23,00	45	71	58,0
06/12/2010	58	19,90	27,00	23,45	29	72	50,5
07/12/2010	58	19,60	27,40	23,50	33	70	51,5
08/12/2010	58	20,30	30,80	25,55	37	73	55,0
09/12/2010	58	15,90	33,20	24,55	29	78	53,5
10/12/2010	58	15,60	30,20	22,90	21	76	48,5
11/12/2010	59	15,00	33,00	24,00	25	85	55,0
12/12/2010	59	15,60	33,20	24,40	21	85	53,0
13/12/2010	59	13,20	30,20	21,70	53	75	64,0
14/12/2010	59	10,60	33,00	21,80	23	76	49,5
15/12/2010	59	10,20	27,00	18,60	22	75	48,5
16/12/2010	59	10,60	30,20	20,40	23	62	42,5
17/12/2010	59	15,40	25,00	20,20	29	70	49,5
18/12/2010	60	12,60	31,00	21,80	35	78	56,5
19/12/2010	60	10,60	33,50	22,05	21	82	51,5
20/12/2010	60	22,50	29,40	25,95	44	81	62,5
21/12/2010	60	21,70	29,70	25,70	42	90	66,0
22/12/2010	60	21,00	28,20	24,60	38	87	62,5
23/12/2010	60	21,00	29,80	25,40	75	90	82,5
24/12/2010	60	23,60	30,00	26,80	37	74	55,5
25/12/2010	61	22,50	34,80	28,65	26	83	54,5
26/12/2010	61	17,30	34,60	25,95	26	83	54,5
27/12/2010	61	17,30	34,80	26,05	31	71	51,0
28/12/2010	61	17,80	28,60	23,20	31	71	51,0
29/12/2010	61	17,80	28,60	23,20	29	71	50,0
30/12/2010	61	17,80	28,60	23,20	29	72	50,5
31/12/2010	61	17,80	28,60	23,20	29	72	50,5
01/01/2011	62	17,80	28,60	23,20	29	76	52,5
02/01/2011	62	17,80	29,10	23,45	29	78	53,5
03/01/2011	62	17,80	29,20	23,50	29	78	53,5
04/01/2011	62	20,40	24,40	22,40	27	80	53,5
05/01/2011	62	17,00	24,00	20,50	29	73	51,0
06/01/2011	62	23,20	35,00	29,10	26	80	53,0
07/01/2011	62	23,60	32,90	28,25	28	78	53,0
08/01/2011	63	23,00	33,30	28,15	27	76	51,5
09/01/2011	63	22,20	36,40	29,30	28	76	52,0
10/01/2011	63	22,20	36,00	29,10	34	80	57,0
11/01/2011	63	21,90	36,40	29,15	37	75	56,0
12/01/2011	63	21,90	30,00	25,95	33	74	53,5
13/01/2011	63	21,60	32,60	27,10	32	81	56,5
14/01/2011	63	20,90	29,00	24,95	33	80	56,5
15/01/2011	64	20,00	31,00	25,50	40	82	61,0

16/01/2011	64	20,90	31,00	25,95	39	83	61,0
17/01/2011	64	23,40	34,10	28,75	25	72	48,5
18/01/2011	64	21,70	24,00	22,85	28	86	57,0
19/01/2011	64	21,40	28,80	25,10	27	83	55,0
20/01/2011	64	22,00	30,90	26,45	28	82	55,0
21/01/2011	64	21,80	31,00	26,40	39	81	60,0
22/01/2011	65	21,00	30,90	25,95	46	78	62,0
23/01/2011	65	21,80	25,40	23,60	60	76	68,0
24/01/2011	65	20,50	28,50	24,50	53	75	64,0
25/01/2011	65	20,00	30,30	25,15	43	82	62,5
26/01/2011	65	20,50	34,40	27,45	36	72	54,0
27/01/2011	65	21,00	34,90	27,95	75	83	79,0
28/01/2011	65	20,50	34,00	27,25	36	70	53,0
29/01/2011	66	21,00	32,60	26,80	41	75	58,0
30/01/2011	66	20,50	34,90	27,70	44	75	59,5
31/01/2011	66	21,00	31,80	26,40	30	72	51,0
01/02/2011	66	22,00	33,50	27,75	28	80	54,0
02/02/2011	66	18,80	30,40	24,60	30	64	47,0
03/02/2011	66	18,00	31,20	24,60	31	65	48,0
04/02/2011	66	18,00	29,80	23,90	42	60	51,0
05/02/2011	67	19,00	32,60	25,80	38	73	55,5
06/02/2011	67	17,50	29,00	23,25	75	80	77,5
07/02/2011	67	19,00	31,00	25,00	37	78	57,5
08/02/2011	67	18,50	31,00	24,75	26	76	51,0
09/02/2011	67	17,40	34,10	25,75	26	76	51,0
10/02/2011	67	16,70	24,00	20,35	31	80	55,5
11/02/2011	67	19,30	28,80	24,05	31	75	53,0
12/02/2011	68	20,50	30,90	25,70	29	74	51,5
13/02/2011	68	21,00	31,00	26,00	29	81	55,0
14/02/2011	68	22,00	30,90	26,45	29	80	54,5
15/02/2011	68	18,80	25,40	22,10	29	82	55,5
16/02/2011	68	18,00	30,30	24,15	29	83	56,0
17/02/2011	68	18,00	34,40	26,20	29	72	50,5
18/02/2011	68	19,00	29,80	24,40	27	86	56,5

Apêndice 4. Peso médio das reprodutoras pesadas por período

Box	Trt	Período	Peso Vivo, kg
1	1	1	3,446
2	3	1	3,493
3	2	1	3,473
4	3	1	3,462
5	1	1	3,451
6	2	1	3,433
7	1	1	3,499
8	2	1	3,429
9	3	1	3,398
10	2	1	3,448
11	1	1	3,455
12	3	1	3,484
13	2	1	3,441
14	3	1	3,512
15	1	1	3,426
16	2	1	3,530
17	1	1	3,467
18	3	1	3,477
19	1	1	3,467
20	3	1	3,451
21	2	1	3,430
22	3	1	3,474
23	1	1	3,448
24	2	1	3,406
25	1	1	3,479
26	3	1	3,487
27	2	1	3,465
28	1	1	3,442
29	2	1	3,465
30	3	1	3,524
1	1	2	3,787
2	3	2	3,814
3	2	2	3,907
4	3	2	3,895
5	1	2	3,708
6	2	2	3,729
7	1	2	3,820
8	2	2	3,867
9	3	2	3,838
10	2	2	3,839
11	1	2	3,808
12	3	2	3,831
13	2	2	3,827
14	3	2	3,892
15	1	2	3,838
16	2	2	3,848
17	1	2	3,814
18	3	2	3,894
19	1	2	3,867

20	3	2	3,859
21	2	2	3,898
22	3	2	3,946
23	1	2	3,790
24	2	2	3,690
25	1	2	3,801
26	3	2	3,873
27	2	2	3,835
28	1	2	3,769
29	2	2	3,888
30	3	2	3,927
1	1	3	3,913
2	3	3	3,902
3	2	3	4,072
4	3	3	3,970
5	1	3	3,830
6	2	3	3,852
7	1	3	4,001
8	2	3	4,111
9	3	3	4,005
10	2	3	3,963
11	1	3	3,969
12	3	3	3,919
13	2	3	4,009
14	3	3	4,014
15	1	3	4,100
16	2	3	4,019
17	1	3	4,041
18	3	3	4,124
19	1	3	4,060
20	3	3	4,038
21	2	3	4,125
22	3	3	4,111
23	1	3	3,929
24	2	3	3,890
25	1	3	3,935
26	3	3	3,939
27	2	3	4,004
28	1	3	3,903
29	2	3	4,031
30	3	3	4,098
31	2	3	4,032
32	3	3	4,035
1	1	4	4,232
2	3	4	4,131
3	2	4	4,264
4	3	4	4,232
5	1	4	4,019
6	2	4	4,012
7	1	4	4,181
8	2	4	4,357

9	3	4	4,153
10	2	4	4,177
11	1	4	4,102
12	3	4	4,094
13	2	4	4,227
14	3	4	4,094
15	1	4	4,235
16	2	4	4,144
17	1	4	4,228
18	3	4	4,082
19	1	4	4,128
20	3	4	4,335
21	2	4	4,217
22	3	4	4,245
23	1	4	4,003
24	2	4	4,066
25	1	4	4,113
26	3	4	3,997
27	2	4	4,113
28	1	4	4,061
29	2	4	4,125
30	3	4	4,286

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 - 25 a 35 Semanas; Período 2 - 36 a 46 Semanas; Período 3 - 47 a 57 Semanas; Período 4 - 58 a 68 Semanas.

Apêndice 5. Produção média de ovos e total de ovos produzidos por período

Box	Trt	Período	Produção diária de ovos, %	Produção de ovos por ave alojada, %	Total de ovos por ave alojada	Total de ovos incubáveis por ave alojada
1	1	1	71,24	70,13	54,00	51,35
2	3	1	72,87	71,82	55,30	52,60
3	2	1	71,96	70,84	54,55	52,15
4	3	1	72,44	71,36	54,95	52,35
5	1	1	72,59	71,49	55,05	52,35
6	2	1	70,98	69,94	53,85	51,30
7	1	1	70,71	69,42	53,45	50,75
8	2	1	71,68	67,79	52,20	49,80
9	3	1	68,55	67,47	51,95	50,50
10	2	1	69,88	68,77	52,95	50,55
11	1	1	70,14	69,09	53,20	51,20
12	3	1	66,98	65,91	50,75	48,70
13	2	1	73,40	72,34	55,70	54,20
14	3	1	70,19	68,70	52,90	51,35
15	1	1	70,83	69,74	53,70	51,10
16	2	1	70,98	69,87	53,80	51,90
17	1	1	69,18	68,12	52,45	50,75
18	3	1	67,35	66,30	51,05	48,95
19	1	1	65,47	58,64	45,15	44,90
20	3	1	69,83	68,70	52,90	51,20
21	2	1	68,44	65,39	50,35	48,30
22	3	1	69,08	63,05	48,55	46,05
23	1	1	69,24	68,18	52,50	50,25
24	2	1	73,52	69,03	53,15	51,45
25	1	1	72,37	71,23	54,85	52,60
26	3	1	71,00	69,87	53,80	51,25
27	2	1	69,37	68,31	52,60	49,95
28	1	1	69,43	68,31	52,60	50,90
29	2	1	68,79	64,94	50,00	47,95
30	3	1	65,95	64,87	49,95	47,80
1	1	2	75,00	75,00	57,75	56,30
2	3	2	76,75	76,75	59,10	58,10
3	2	2	77,53	77,53	59,70	58,35
4	3	2	75,50	73,77	56,80	55,25
5	1	2	76,23	74,22	57,15	55,55
6	2	2	77,52	75,84	58,40	57,55
7	1	2	76,10	70,71	54,45	53,00
8	2	2	72,71	68,18	52,50	51,75
9	3	2	74,68	74,68	57,50	56,75
10	2	2	74,16	70,45	54,25	53,15
11	1	2	73,71	70,13	54,00	52,70
12	3	2	72,01	72,01	55,45	54,10
13	2	2	74,87	73,25	56,40	55,35
14	3	2	73,96	70,26	54,10	52,70
15	1	2	72,73	72,73	56,00	54,20
16	2	2	69,48	69,48	53,50	52,45
17	1	2	67,54	71,30	54,90	52,53
18	3	2	71,53	69,68	53,65	52,75

19	1	2	72,52	67,60	52,05	50,92
20	3	2	76,23	74,22	57,15	56,15
21	2	2	72,31	66,95	51,55	50,45
22	3	2	75,25	67,73	52,15	50,90
23	1	2	74,65	72,79	56,05	54,95
24	2	2	75,12	71,36	54,95	54,10
25	1	2	72,53	72,53	55,85	54,75
26	3	2	74,38	73,51	56,60	55,55
27	2	2	73,12	73,12	56,30	55,10
28	1	2	72,79	72,79	56,05	55,45
29	2	2	76,17	69,03	53,15	52,20
30	3	2	70,16	68,64	52,85	52,05
1	1	3	64,61	64,61	49,75	48,95
2	3	3	65,52	65,52	50,45	49,60
3	2	3	64,61	64,61	49,75	48,85
4	3	3	64,35	60,52	46,60	45,25
5	1	3	66,64	63,31	48,75	47,75
6	2	3	65,28	62,01	47,75	47,20
7	1	3	64,29	57,86	44,55	43,65
8	2	3	62,55	56,30	43,35	42,05
9	3	3	63,12	63,12	48,60	47,80
10	2	3	65,81	61,17	47,10	46,30
11	1	3	61,62	55,45	42,70	41,80
12	3	3	63,17	61,95	47,70	46,90
13	2	3	62,67	59,22	45,60	44,85
14	3	3	65,69	62,40	48,05	47,50
15	1	3	63,62	58,31	44,90	43,95
16	2	3	61,75	61,75	47,55	46,60
17	1	3	59,95	56,95	43,85	42,70
18	3	3	67,20	57,73	44,45	43,55
19	1	3	65,39	55,58	42,80	41,85
20	3	3	62,01	58,05	44,70	43,80
21	2	3	62,13	59,03	45,45	44,90
22	3	3	68,18	61,36	47,25	46,65
23	1	3	66,44	61,88	47,65	47,10
24	2	3	63,44	60,19	46,35	45,50
25	1	3	63,49	61,82	47,60	46,40
26	3	3	66,23	62,92	48,45	47,60
27	2	3	66,04	66,04	50,85	50,30
28	1	3	64,42	64,42	49,60	49,00
29	2	3	67,77	56,69	43,65	42,95
30	3	3	62,54	59,42	45,75	45,30
1	1	4	57,16	54,48	41,95	40,95
2	3	4	53,90	51,04	39,30	38,25
3	2	4	55,65	51,10	39,35	38,80
4	3	4	51,30	43,57	33,55	32,70
5	1	4	59,44	43,31	33,35	29,03
6	2	4	57,37	51,56	39,70	38,80
7	1	4	55,19	47,34	36,45	30,40
8	2	4	54,31	46,17	35,55	34,60
9	3	4	54,72	51,95	40,00	39,30

10	2	4	58,13	49,29	37,95	37,20
11	1	4	58,95	50,45	38,85	37,65
12	3	4	56,57	50,97	39,25	38,50
13	2	4	52,98	45,52	35,05	34,05
14	3	4	58,94	51,95	40,00	39,40
15	1	4	53,53	42,73	32,90	32,45
16	2	4	53,79	51,10	39,35	38,45
17	1	4	50,33	44,03	33,90	33,40
18	3	4	59,26	48,12	37,05	36,60
19	1	4	55,87	45,32	34,90	34,05
20	3	4	53,99	44,61	34,35	33,90
21	2	4	55,82	50,78	39,10	38,45
22	3	4	58,69	53,25	41,00	40,40
23	1	4	59,49	53,57	41,25	40,90
24	2	4	57,74	49,81	38,35	37,60
25	1	4	54,09	48,77	37,55	36,60
26	3	4	60,20	54,42	41,90	41,15
27	2	4	61,08	57,79	44,50	44,10
28	1	4	59,11	56,17	43,25	42,90
29	2	4	58,47	44,61	34,35	33,45
30	3	4	53,47	48,12	37,05	36,55

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 - 25 a 35 Semanas; Período 2 - 36 a 46 Semanas; Período 3 - 47 a 57 Semanas; Período 4 - 58 a 68 Semanas.

Apêndice 6. Peso de ovo e parâmetros de qualidade da casca do ovo por período

Box	Trt	Período	Peso Ovo, g	Gravidade específica, g/ml	Peso relativo da casca, %	Espessura da casca, µm
1	1	1	64,00	1090,00	9,33	378,07
2	3	1	63,08	1087,31	9,55	396,00
3	2	1	65,09	1085,31	9,21	387,07
4	3	1	65,97	1088,53	9,73	391,27
5	1	1	66,04	1085,71	9,70	382,00
6	2	1	63,33	1084,17	9,52	382,07
7	1	1	64,60	1088,67	9,70	391,27
8	2	1	66,25	1087,86	9,45	397,87
9	3	1	66,71	1085,71	9,59	396,80
10	2	1	65,83	1088,33	9,15	389,20
11	1	1	64,96	1086,15	9,20	382,80
12	3	1	65,03	1086,88	9,25	386,40
13	2	1	64,50	1085,83	9,47	383,00
14	3	1	65,54	1086,79	9,30	393,20
15	1	1	65,21	1085,38	9,05	384,80
16	2	1	66,86	1087,14	9,50	384,80
17	1	1	66,61	1087,22	9,38	393,73
18	3	1	64,79	1087,92	9,26	390,67
19	1	1	66,28	1087,22	9,58	388,87
20	3	1	65,86	1087,50	9,97	386,53
21	2	1	65,58	1088,46	10,03	384,20
22	3	1	65,71	1088,33	9,52	390,93
23	1	1	65,65	1085,38	8,76	390,60
24	2	1	62,70	1085,50	9,18	386,53
25	1	1	64,00	1087,19	8,82	389,20
26	3	1	62,46	1087,31	9,27	382,27
27	2	1	62,44	1086,88	9,40	394,87
28	1	1	66,54	1087,08	8,80	394,13
29	2	1	64,38	1089,58	9,62	388,73
30	3	1	64,70	1087,78	9,69	391,93
1	1	2	69,13	1085,91	9,79	371,07
2	3	2	69,85	1085,45	9,46	382,60
3	2	2	72,81	1082,86	9,34	389,40
4	3	2	68,82	1084,00	9,20	387,33
5	1	2	70,32	1085,00	8,41	384,13
6	2	2	71,03	1085,29	9,20	383,47
7	1	2	67,79	1084,09	9,60	380,67
8	2	2	70,72	1086,11	9,66	390,00
9	3	2	69,29	1086,07	9,04	387,07
10	2	2	70,54	1087,50	9,83	381,07
11	1	2	68,89	1083,33	9,53	389,27
12	3	2	69,58	1083,33	8,98	380,13
13	2	2	69,44	1085,56	9,04	395,80
14	3	2	71,04	1088,00	9,43	382,53
15	1	2	68,36	1088,44	9,02	373,93
16	2	2	72,44	1082,22	8,70	383,53
17	1	2	68,96	1087,86	8,94	393,13

18	3	2	67,33	1088,33	9,45	396,27
19	1	2	72,38	1086,36	9,28	398,47
20	3	2	68,70	1088,00	9,76	385,53
21	2	2	69,38	1084,44	9,51	377,67
22	3	2	68,67	1086,11	9,52	387,33
23	1	2	69,50	1087,69	8,97	389,33
24	2	2	67,90	1084,55	9,53	372,60
25	1	2	69,90	1084,44	9,48	370,93
26	3	2	67,68	1087,31	9,17	378,13
27	2	2	70,63	1088,00	9,48	386,33
28	1	2	69,78	1085,36	9,10	370,47
29	2	2	71,08	1088,64	9,43	375,27
30	3	2	69,56	1089,58	9,66	388,13
1	1	3	72,04	1085,00	9,05	384,13
2	3	3	71,17	1085,42	8,94	380,00
3	2	3	73,91	1083,64	8,63	383,73
4	3	3	72,04	1083,08	8,53	378,27
5	1	3	72,64	1083,18	8,61	388,67
6	2	3	73,20	1085,00	8,86	387,87
7	1	3	72,61	1084,44	8,76	386,13
8	2	3	76,14	1083,57	8,98	387,40
9	3	3	74,50	1085,00	8,90	385,60
10	2	3	74,65	1085,50	8,89	377,27
11	1	3	73,08	1082,50	8,72	379,33
12	3	3	74,42	1083,75	8,81	380,87
13	2	3	70,13	1082,50	8,65	372,20
14	3	3	74,72	1085,00	9,38	388,87
15	1	3	72,18	1085,45	9,16	385,67
16	2	3	74,28	1082,22	9,00	385,53
17	1	3	72,96	1084,29	9,01	379,87
18	3	3	70,50	1085,83	9,25	390,60
19	1	3	73,30	1083,50	8,62	376,67
20	3	3	72,70	1087,00	9,40	389,33
21	2	3	71,11	1083,33	8,97	380,80
22	3	3	71,77	1084,62	8,28	385,33
23	1	3	71,55	1083,18	8,25	378,20
24	2	3	73,30	1085,00	8,32	386,80
25	1	3	72,33	1084,17	8,74	378,67
26	3	3	71,56	1083,89	8,91	386,87
27	2	3	73,05	1084,09	8,68	377,47
28	1	3	72,39	1085,00	8,40	372,67
29	2	3	71,42	1084,17	8,94	386,93
30	3	3	72,73	1086,82	9,18	384,93
1	1	4	71,20	1082,00	8,56	376,93
2	3	4	71,94	1085,00	8,95	373,87
3	2	4	74,80	1086,00	8,60	372,56
4	3	4	73,07	1082,14	9,37	379,13
5	1	4	73,33	1082,50	8,70	380,13
6	2	4	74,93	1083,57	8,93	380,00
7	1	4	77,20	1084,00	8,98	372,93
8	2	4	77,00	1082,14	8,56	382,53

9	3	4	77,41	1082,78	8,80	393,47
10	2	4	76,00	1084,38	8,86	380,60
11	1	4	75,33	1085,00	8,82	383,27
12	3	4	75,00	1085,71	8,61	389,00
13	2	4	72,25	1086,25	9,05	392,50
14	3	4	73,44	1083,33	9,08	380,40
15	1	4	75,50	1085,00	8,69	395,67
16	2	4	73,50	1082,50	8,85	387,73
17	1	4	73,28	1082,78	8,64	370,27
18	3	4	73,86	1080,71	9,13	385,53
19	1	4	74,00	1084,29	8,77	373,33
20	3	4	73,60	1086,00	9,07	392,07
21	2	4	77,00	1083,57	8,80	382,80
22	3	4	74,50	1085,56	8,67	390,87
23	1	4	73,65	1084,00	8,40	376,53
24	2	4	72,88	1083,00	8,55	385,40
25	1	4	73,00	1085,00	8,48	374,27
26	3	4	73,38	1085,00	9,07	384,07
27	2	4	73,79	1082,86	8,76	382,07
28	1	4	74,29	1085,00	8,65	373,20
29	2	4	73,75	1084,38	8,55	387,93
30	3	4	75,31	1085,83	8,62	383,20

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 - 35 Semanas; Período 2 - 45 Semanas; Período 3 - 55 Semanas; Período 4 - 65 Semanas.

Apêndice 7. Fertilidade, eclodibilidadese pintos por ave alojada das por período

Box	Trt	Período	Fertilidade, %	Eclodibilidade, %	Eclodibilidade dos ovos férteis, %	Pintos por ave alojada
1	1	1	84,32	79,15	94,83	40,64
2	3	1	94,44	90,10	95,66	47,39
3	2	1	81,16	76,98	95,81	40,14
4	3	1	94,20	87,48	93,93	45,80
5	1	1	93,50	79,56	88,12	41,65
6	2	1	96,86	87,88	91,02	45,08
7	1	1	96,31	81,67	88,75	41,45
8	2	1	97,71	86,88	92,10	43,27
9	3	1	95,17	87,06	91,89	43,96
10	2	1	89,65	80,12	90,48	40,50
11	1	1	97,18	85,12	89,70	43,58
12	3	1	99,33	89,18	92,76	43,43
13	2	1	95,77	89,20	94,06	48,35
14	3	1	92,50	88,99	97,07	45,69
15	1	1	94,42	83,39	90,44	42,61
16	2	1	89,64	80,67	92,25	41,87
17	1	1	88,41	79,19	92,21	40,19
18	3	1	92,60	84,58	91,98	41,40
19	1	1	99,62	90,13	90,50	40,47
20	3	1	89,55	86,45	96,90	44,26
21	2	1	97,95	89,52	92,19	43,24
22	3	1	94,70	87,63	94,81	40,35
23	1	1	96,63	87,65	92,19	44,04
24	2	1	92,13	83,33	92,01	42,87
25	1	1	91,39	81,90	92,35	43,08
26	3	1	93,75	86,44	93,28	44,30
27	2	1	90,86	83,92	93,64	41,92
28	1	1	87,25	83,74	96,61	42,63
29	2	1	92,69	83,19	91,84	39,89
30	3	1	95,68	87,99	92,87	42,06
1	1	2	79,09	71,51	93,89	40,26
2	3	2	89,41	83,18	93,77	48,33
3	2	2	92,15	78,17	87,41	45,61
4	3	2	88,52	80,96	92,44	44,73
5	1	2	94,21	79,29	86,41	44,05
6	2	2	97,24	85,88	90,09	49,42
7	1	2	94,58	77,21	84,60	40,92
8	2	2	83,09	78,04	94,95	40,39
9	3	2	83,13	76,88	95,33	43,63
10	2	2	92,90	80,64	89,28	42,86
11	1	2	96,41	84,96	88,56	44,78
12	3	2	94,98	85,23	91,69	46,11
13	2	2	97,44	83,14	90,04	46,02
14	3	2	95,19	87,03	91,84	45,86
15	1	2	92,57	80,62	94,43	43,70
16	2	2	88,15	82,48	94,33	43,26
17	1	2	99,71	77,40	78,20	40,66
18	3	2	90,65	77,32	88,14	40,79

19	1	2	99,09	80,79	82,65	41,14
20	3	2	84,49	76,66	92,18	43,05
21	2	2	95,33	85,14	92,89	42,95
22	3	2	93,02	81,92	88,90	41,69
23	1	2	93,05	83,22	91,52	45,73
24	2	2	86,34	75,96	91,29	41,09
25	1	2	86,90	78,45	93,14	42,95
26	3	2	91,43	84,27	95,73	46,81
27	2	2	93,70	84,09	96,36	46,33
28	1	2	98,68	87,63	90,30	48,59
29	2	2	94,84	82,56	93,97	43,10
30	3	2	96,30	84,27	89,42	43,86
1	1	3	86,08	77,67	93,55	38,02
2	3	3	88,70	80,92	93,67	40,14
3	2	3	82,15	73,38	93,16	35,85
4	3	3	97,50	85,49	89,77	38,68
5	1	3	94,66	79,00	88,07	37,72
6	2	3	85,40	70,41	85,64	33,23
7	1	3	88,42	77,03	88,60	33,62
8	2	3	99,20	86,96	87,76	36,57
9	3	3	95,55	82,20	89,56	39,29
10	2	3	95,82	77,92	83,97	36,08
11	1	3	94,59	80,78	89,52	33,76
12	3	3	98,37	79,55	81,17	37,31
13	2	3	97,65	84,90	92,12	38,08
14	3	3	97,59	83,26	93,87	39,55
15	1	3	93,36	79,00	93,21	34,72
16	2	3	87,89	79,90	96,97	37,23
17	1	3	99,11	80,55	81,44	34,40
18	3	3	95,07	81,20	90,66	35,36
19	1	3	98,83	79,39	81,76	33,22
20	3	3	93,24	80,47	87,92	35,25
21	2	3	97,62	84,23	88,76	37,82
22	3	3	92,01	78,09	87,48	36,43
23	1	3	95,65	83,46	89,17	39,31
24	2	3	92,69	84,15	91,46	38,29
25	1	3	91,31	74,73	88,62	34,67
26	3	3	94,73	86,03	93,62	40,95
27	2	3	92,58	68,67	83,11	34,54
28	1	3	91,07	80,09	89,02	39,24
29	2	3	94,58	81,02	89,63	34,80
30	3	3	92,82	80,62	87,80	36,52
1	1	4	88,61	74,59	87,31	30,55
2	3	4	91,81	79,08	90,21	30,25
3	2	4	91,28	78,67	87,39	30,52
4	3	4	85,45	74,48	89,03	24,35
5	1	4	84,93	73,44	94,50	21,32
6	2	4	70,73	60,26	91,23	23,38
7	1	4	91,40	69,54	82,07	21,14
8	2	4	85,07	71,68	88,24	24,80
9	3	4	84,97	65,15	85,36	25,60

10	2	4	91,82	76,88	88,09	28,60
11	1	4	93,16	80,79	87,64	30,42
12	3	4	93,66	74,07	80,41	28,52
13	2	4	93,10	83,72	94,84	28,51
14	3	4	86,62	67,73	91,11	26,69
15	1	4	94,97	69,19	79,48	22,45
16	2	4	87,48	73,03	95,55	28,08
17	1	4	99,71	79,29	88,05	26,48
18	3	4	95,91	73,20	80,29	26,79
19	1	4	93,93	73,29	79,36	24,95
20	3	4	97,29	77,70	83,92	26,34
21	2	4	91,03	66,79	82,11	25,68
22	3	4	72,88	65,00	92,12	26,26
23	1	4	87,33	77,21	89,89	31,58
24	2	4	95,76	74,42	81,94	27,98
25	1	4	89,52	70,38	85,41	25,76
26	3	4	86,48	68,32	81,85	28,11
27	2	4	74,19	65,44	93,69	28,86
28	1	4	84,72	56,18	79,53	24,10
29	2	4	93,76	72,05	85,97	24,10
30	3	4	87,76	75,30	90,94	27,52

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 – 45 a 50 Semanas; Período 2 – 51 a 56 Semanas; Período 3 – 57 a 62 Semanas; Período 4 – 63 a 68 Semanas.

Apêndice 8. Mortalidade embrionária: Embriodiagnóstico dos ovos não eclodidos por período

Box	Trt	Período	Inicial, %	Intermediária, %	Final, %	Bicados, %
1	1	1	1,15	0,15	1,30	2,57
2	3	1	1,29	0,00	1,20	1,85
3	2	1	0,60	0,60	2,39	0,60
4	3	1	1,21	0,42	3,03	1,41
5	1	1	2,01	0,26	5,15	4,46
6	2	1	0,63	0,00	4,20	4,15
7	1	1	3,16	1,00	3,39	3,69
8	2	1	0,81	1,79	4,14	1,16
9	3	1	1,27	0,61	4,36	1,88
10	2	1	1,59	0,79	3,97	3,17
11	1	1	2,52	1,16	3,05	3,56
12	3	1	1,94	0,66	3,48	1,16
13	2	1	1,73	0,00	3,07	1,14
14	3	1	0,00	1,18	1,16	0,59
15	1	1	2,00	0,00	3,41	4,15
16	2	1	0,00	1,29	1,93	4,53
17	1	1	2,30	0,19	2,30	3,00
18	3	1	0,60	0,00	4,97	2,45
19	1	1	0,63	1,88	4,07	2,92
20	3	1	0,61	0,00	1,88	0,61
21	2	1	1,85	0,68	2,86	2,41
22	3	1	1,27	0,15	2,50	1,27
23	1	1	1,89	0,62	3,54	1,76
24	2	1	1,15	0,57	4,19	2,08
25	1	1	2,36	0,60	2,88	1,81
26	3	1	1,85	0,00	4,25	0,62
27	2	1	2,32	0,58	1,16	2,31
28	1	1	1,72	0,12	1,56	0,00
29	2	1	0,11	0,00	6,70	1,35
30	3	1	1,75	0,00	3,47	1,90
1	1	2	2,43	0,00	1,98	1,70
2	3	2	2,11	0,48	2,23	1,41
3	2	2	2,98	0,58	5,56	3,47
4	3	2	1,52	0,00	3,19	2,85
5	1	2	2,33	1,30	6,95	3,00
6	2	2	1,30	0,00	5,29	3,32
7	1	2	4,92	1,96	3,96	4,56
8	2	2	0,65	0,00	1,29	3,11
9	3	2	2,97	0,00	1,70	0,00
10	2	2	1,54	0,00	6,06	3,13
11	1	2	2,34	0,50	4,85	3,76
12	3	2	2,68	0,77	2,79	2,07
13	2	2	2,81	0,00	3,61	3,53
14	3	2	2,91	1,32	1,90	2,04
15	1	2	1,00	0,00	2,54	2,02
16	2	2	1,59	0,63	2,21	1,25
17	1	2	3,98	0,69	9,09	8,03
18	3	2	2,05	0,00	4,82	4,99

19	1	2	4,00	0,35	6,16	6,83
20	3	2	1,20	0,00	2,09	4,54
21	2	2	0,62	1,23	2,48	2,77
22	3	2	2,67	0,67	5,05	2,71
23	1	2	2,57	1,27	3,02	1,62
24	2	2	1,55	0,00	3,35	3,81
25	1	2	3,44	0,00	2,63	0,79
26	3	2	0,71	0,15	1,30	2,10
27	2	2	1,17	0,00	0,99	1,49
28	1	2	1,92	1,14	3,27	3,37
29	2	2	0,00	0,00	6,03	0,00
30	3	2	1,90	0,00	6,64	2,04
1	1	3	2,42	0,69	2,53	0,81
2	3	3	1,67	1,67	2,28	0,72
3	2	3	1,92	0,89	3,38	0,64
4	3	3	4,79	0,96	3,89	0,60
5	1	3	3,06	0,73	5,22	2,92
6	2	3	4,72	1,06	3,48	5,09
7	1	3	3,17	1,70	6,52	0,00
8	2	3	3,86	0,74	5,87	1,78
9	3	3	2,57	0,50	4,53	2,84
10	2	3	1,46	0,19	12,29	2,08
11	1	3	3,36	1,24	1,94	3,94
12	3	3	4,23	1,56	7,56	5,47
13	2	3	0,26	1,22	2,59	3,81
14	3	3	0,85	0,00	2,73	2,55
15	1	3	2,61	0,27	2,15	1,76
16	2	3	0,00	0,00	1,18	1,86
17	1	3	1,77	0,62	7,77	8,40
18	3	3	1,72	0,88	4,25	2,50
19	1	3	3,77	0,19	9,72	4,56
20	3	3	3,60	0,09	5,49	2,90
21	2	3	3,17	0,00	4,54	3,52
22	3	3	2,27	0,76	5,92	3,57
23	1	3	2,90	1,18	4,79	1,96
24	2	3	1,74	0,00	4,90	1,90
25	1	3	5,32	0,74	3,06	2,26
26	3	3	2,19	0,00	2,80	1,39
27	2	3	1,75	1,75	9,87	3,51
28	1	3	2,11	0,00	7,68	1,19
29	2	3	3,18	0,60	6,00	0,60
30	3	3	6,12	0,00	5,47	0,60
1	1	4	5,00	0,19	5,83	1,67
2	3	4	4,47	0,11	3,21	2,00
3	2	4	3,24	0,96	4,55	3,85
4	3	4	3,84	1,64	5,49	0,00
5	1	4	1,43	0,21	2,43	1,43
6	2	4	2,30	0,00	3,08	3,39
7	1	4	4,41	0,98	10,27	2,27
8	2	4	5,97	1,25	3,71	0,82
9	3	4	6,45	0,47	5,86	1,86

10	2	4	3,97	0,19	6,05	1,70
11	1	4	2,19	0,91	8,36	0,91
12	3	4	5,85	2,33	4,97	6,44
13	2	4	2,11	1,02	1,01	1,02
14	3	4	2,95	0,53	4,13	1,28
15	1	4	6,32	2,39	6,40	5,41
16	2	4	1,68	0,32	0,73	1,73
17	1	4	4,57	0,00	5,39	1,98
18	3	4	5,00	2,00	6,00	6,71
19	1	4	10,00	2,14	5,00	3,50
20	3	4	3,51	2,38	8,58	1,61
21	2	4	5,97	0,00	5,59	6,33
22	3	4	2,55	0,00	2,29	3,04
23	1	4	3,74	0,94	2,11	3,32
24	2	4	6,48	0,00	7,19	4,39
25	1	4	3,23	1,04	6,88	3,44
26	3	4	2,60	2,58	8,99	3,98
27	2	4	1,22	1,19	2,10	1,80
28	1	4	5,61	4,19	5,84	4,84
29	2	4	3,09	0,81	5,11	5,01
30	3	4	4,18	1,06	1,69	2,13

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 – 45 a 50 Semanas; Período 2 – 51 a 56 Semanas; Período 3 – 57 a 62 Semanas; Período 4 – 63 a 68 Semanas.

Apêndice 9. Classificação de pintinhos, comprimento e peso médio por período

Box	Trt	Período	Pintos de primeira, %	Refugos, %	Probl. Locomotores, %	Probl. de Umbigo, %	Comp. pintinho, cm	Peso médio pintinho, g
1	1	1	87,50	0,00	1,25	11,25	19,33	49,28
2	3	1	93,75	0,00	0,00	3,75	19,14	49,63
3	2	1	93,13	1,88	0,00	5,00	19,41	50,08
4	3	1	91,34	1,25	0,59	6,57	19,34	49,40
5	1	1	85,76	2,10	2,72	9,42	19,47	50,50
6	2	1	87,27	1,85	1,31	9,57	19,56	49,96
7	1	1	94,44	0,00	1,85	3,70	19,14	48,11
8	2	1	90,51	0,63	0,68	8,17	19,40	49,22
9	3	1	85,38	2,00	1,41	11,21	19,58	51,01
10	2	1	88,70	0,00	1,79	9,51	19,44	49,70
11	1	1	89,50	0,67	0,00	7,33	19,21	49,48
12	3	1	92,80	1,37	0,00	5,83	19,44	51,16
13	2	1	92,28	0,67	0,00	7,05	19,44	49,13
14	3	1	93,33	0,60	0,61	5,46	19,65	50,10
15	1	1	89,66	1,72	0,00	8,62	19,52	50,81
16	2	1	88,08	2,09	0,00	9,83	19,26	50,77
17	1	1	78,23	8,47	0,00	13,90	19,19	51,73
18	3	1	91,84	3,41	2,02	2,74	19,51	48,95
19	1	1	85,58	3,30	1,30	9,82	19,31	50,37
20	3	1	95,38	0,00	0,00	4,62	19,31	48,66
21	2	1	90,18	7,84	0,60	1,39	19,50	51,49
22	3	1	91,26	0,67	0,00	8,08	19,33	49,39
23	1	1	93,35	1,85	0,68	4,11	19,64	49,06
24	2	1	88,65	1,93	0,65	8,78	19,17	49,17
25	1	1	91,24	3,43	0,00	5,33	19,28	52,00
26	3	1	91,46	1,97	0,64	5,92	19,50	48,34
27	2	1	88,65	0,00	0,61	10,74	19,44	50,78
28	1	1	91,23	0,00	1,18	7,59	19,20	48,65
29	2	1	89,76	1,39	1,48	7,38	19,18	49,30
30	3	1	86,30	1,27	0,67	11,76	19,31	49,67
1	1	2	88,98	4,58	0,63	5,80	19,55	50,94
2	3	2	91,37	3,47	0,00	5,16	19,98	50,22
3	2	2	93,59	0,81	0,00	5,61	19,61	51,25
4	3	2	87,04	4,21	0,00	8,75	19,75	50,05
5	1	2	85,89	3,11	0,00	10,99	19,67	51,91
6	2	2	83,41	3,95	0,00	12,64	19,75	51,52
7	1	2	86,96	0,00	2,17	10,87	19,40	51,03
8	2	2	86,18	4,53	0,79	8,50	19,60	51,84
9	3	2	89,19	2,50	0,85	7,46	19,65	52,45
10	2	2	89,69	3,91	0,00	6,41	19,53	51,25
11	1	2	94,57	1,61	0,74	3,08	19,54	51,75
12	3	2	93,34	0,68	0,82	5,16	19,71	51,39
13	2	2	90,86	2,11	0,00	7,03	19,47	50,46
14	3	2	87,95	7,04	0,00	5,01	19,74	52,38
15	1	2	82,83	2,50	7,50	7,17	19,54	53,09
16	2	2	85,73	4,86	0,85	8,56	19,47	52,54
17	1	2	81,82	12,73	0,00	5,45	19,27	52,65

18	3	2	78,91	15,22	0,00	5,86	19,48	51,14
19	1	2	81,68	4,69	0,85	12,78	19,25	51,85
20	3	2	86,09	1,74	0,86	11,31	19,34	51,22
21	2	2	92,28	2,24	0,83	4,65	19,27	52,98
22	3	2	88,22	3,13	0,00	8,66	19,29	51,12
23	1	2	87,83	6,14	1,43	4,60	19,47	51,14
24	2	2	89,21	3,60	2,78	4,42	19,68	50,48
25	1	2	85,02	6,20	0,72	8,05	19,32	50,78
26	3	2	86,78	7,27	0,70	5,24	19,51	51,29
27	2	2	84,75	10,17	0,00	5,08	19,44	52,68
28	1	2	83,76	6,26	0,77	9,22	19,52	51,40
29	2	2	71,43	16,07	0,00	12,50	19,25	52,16
30	3	2	92,67	1,50	0,00	5,83	19,54	52,43
1	1	3	86,13	5,83	0,00	8,04	19,67	49,02
2	3	3	90,15	1,89	0,64	7,32	19,83	50,74
3	2	3	89,75	0,75	1,49	8,01	19,67	52,72
4	3	3	87,82	2,03	1,06	9,09	19,85	50,94
5	1	3	81,24	3,71	0,98	14,07	19,64	52,21
6	2	3	85,20	5,89	0,00	8,92	19,77	52,69
7	1	3	71,43	2,38	0,00	26,19	19,19	50,72
8	2	3	80,72	2,10	1,69	15,49	19,66	52,93
9	3	3	84,42	4,51	1,02	10,05	19,74	53,08
10	2	3	84,92	3,24	0,72	11,12	19,63	52,16
11	1	3	94,36	0,00	0,77	4,87	19,41	52,22
12	3	3	87,14	1,43	2,86	8,57	19,64	50,49
13	2	3	88,79	0,00	2,14	9,07	19,54	50,57
14	3	3	85,16	3,34	1,95	9,55	19,97	52,95
15	1	3	77,71	2,47	4,00	15,82	19,77	52,79
16	2	3	84,82	0,68	2,05	12,44	19,65	52,69
17	1	3	77,70	10,00	0,00	12,30	19,27	51,83
18	3	3	84,13	5,16	1,47	9,23	19,74	51,10
19	1	3	72,51	3,17	1,45	22,87	19,00	52,75
20	3	3	82,92	5,88	0,93	10,27	19,59	52,74
21	2	3	76,14	8,74	0,00	15,12	19,30	53,77
22	3	3	69,17	12,94	0,00	17,88	19,43	51,17
23	1	3	82,84	4,15	0,00	13,01	19,69	52,97
24	2	3	78,76	5,25	1,49	14,50	19,84	52,75
25	1	3	82,63	3,65	0,00	13,72	19,51	51,04
26	3	3	83,77	3,73	0,00	12,50	19,50	50,70
27	2	3	80,95	0,00	0,00	19,05	19,46	50,86
28	1	3	85,84	1,75	2,60	9,81	19,78	51,22
29	2	3	81,21	9,58	0,00	9,21	19,48	51,70
30	3	3	87,27	6,22	0,67	5,85	19,65	52,67
1	1	4	80,59	0,78	0,78	17,85	19,80	51,09
2	3	4	89,08	0,82	0,00	10,10	19,95	51,66
3	2	4	87,32	0,00	2,64	10,04	19,71	51,80
4	3	4	92,16	0,00	1,47	6,37	19,76	51,14
5	1	4	79,72	6,36	0,00	13,92	19,74	51,63
6	2	4	89,36	0,00	0,00	10,64	19,86	51,35
7	1	4	83,41	0,00	1,52	15,08	19,42	51,18
8	2	4	74,19	9,95	2,49	13,37	19,62	52,56

9	3	4	86,15	3,52	1,02	9,31	19,88	52,66
10	2	4	81,50	2,85	2,68	12,97	19,72	51,67
11	1	4	90,48	0,00	0,79	8,73	19,61	50,60
12	3	4	92,31	0,00	0,00	7,69	19,66	51,81
13	2	4	83,28	1,94	1,94	12,83	19,61	50,99
14	3	4	80,10	5,72	2,00	12,17	19,81	53,19
15	1	4	83,33	5,56	1,85	9,26	19,60	52,84
16	2	4	84,03	1,22	0,96	13,79	19,42	51,74
17	1	4	75,92	2,45	2,45	19,18	19,56	51,97
18	3	4	74,36	0,00	0,00	25,64	19,50	50,70
19	1	4	78,13	3,13	0,00	18,75	19,13	53,09
20	3	4	76,25	6,04	3,13	14,58	19,43	53,77
21	2	4	83,71	2,39	1,92	11,98	19,41	50,91
22	3	4	79,40	2,68	4,23	13,69	19,38	51,43
23	1	4	80,96	6,90	2,03	10,11	19,65	51,99
24	2	4	77,43	10,51	4,19	7,88	19,72	53,35
25	1	4	86,94	2,18	1,85	9,03	19,58	51,31
26	3	4	81,42	4,55	0,70	13,33	19,47	49,71
27	2	4	85,14	2,70	0,68	11,49	19,52	52,00
28	1	4	90,00	4,00	0,00	6,00	19,27	52,13
29	2	4	82,46	5,26	0,00	12,28	19,42	51,55
30	3	4	85,92	3,75	0,00	10,33	19,48	51,64

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 – 45 a 50 Semanas; Período 2 – 51 a 56 Semanas; Período 3 – 57 a 62 Semanas; Período 4 – 63 a 68 Semanas.

Apêndice 10. Composição mineral dos ovos por período (Gema + albúmen)

Box	Trt	Período	Zn, ppm	Mn, ppm	Cu, ppm
1	1	1	54,05	0,58	2,11
2	3	1	52,94	1,08	2,52
3	2	1	54,27	0,71	2,41
4	3	1	62,44	1,34	2,15
5	1	1	57,17	1,26	2,39
6	2	1	ND	ND	ND
7	1	1	51,46	0,79	1,63
8	2	1	64,44	1,12	1,77
9	3	1	51,97	0,84	2,13
10	2	1	56,64	1,10	2,43
11	1	1	49,05	0,73	ND
12	3	1	62,27	1,47	2,03
13	2	1	58,09	1,13	2,24
14	3	1	55,00	0,74	2,10
15	1	1	58,43	0,87	1,48
16	2	1	52,18	0,72	1,69
17	1	1	57,72	0,82	2,13
18	3	1	52,38	0,96	2,87
19	1	1	59,80	1,44	2,10
20	3	1	56,09	1,25	2,04
21	2	1	57,58	0,72	1,97
22	3	1	53,36	0,94	1,55
23	1	1	53,05	0,72	2,06
24	2	1	57,02	0,82	2,69
25	1	1	55,99	0,71	2,65
26	3	1	57,42	0,85	2,10
27	2	1	ND	0,74	1,77
28	1	1	55,91	1,05	1,43
29	2	1	56,30	1,10	2,24
30	3	1	55,61	1,12	1,47
1	1	2	56,40	1,79	2,07
2	3	2	58,39	ND	ND
3	2	2	61,37	1,20	2,31
4	3	2	58,39	2,22	2,28
5	1	2	66,34	1,41	2,49
6	2	2	57,35	1,11	1,94
7	1	2	53,85	1,03	2,38
8	2	2	62,57	1,27	2,41
9	3	2	57,46	0,44	1,73
10	2	2	57,50	ND	ND
11	1	2	59,78	1,13	2,73
12	3	2	57,19	1,15	2,13
13	2	2	55,31	ND	2,09
14	3	2	55,45	0,60	2,95
15	1	2	53,02	1,17	1,74
16	2	2	57,50	ND	ND
17	1	2	56,40	2,28	2,96
18	3	2	60,69	0,83	1,80
19	1	2	53,52	1,13	1,80

20	3	2	55,45	1,18	2,43
21	2	2	46,56	1,08	2,16
22	3	2	58,46	1,04	2,36
23	1	2	54,75	0,95	2,69
24	2	2	57,50	ND	ND
25	1	2	54,40	1,48	1,19
26	3	2	53,46	1,26	2,14
27	2	2	63,60	1,47	2,49
28	1	2	55,59	0,84	2,00
29	2	2	57,50	1,85	2,40
30	3	2	69,59	2,87	2,19
1	1	3	59,71	1,64	2,29
2	3	3	ND	1,68	2,59
3	2	3	58,25	1,83	1,83
4	3	3	60,86	1,77	1,96
5	1	3	57,17	1,81	2,02
6	2	3	51,25	1,60	2,76
7	1	3	52,70	1,78	2,28
8	2	3	58,60	1,91	1,88
9	3	3	55,24	1,76	2,67
10	2	3	ND	ND	ND
11	1	3	57,00	1,94	2,54
12	3	3	57,28	1,81	2,40
13	2	3	58,08	2,09	2,31
14	3	3	55,78	1,58	2,07
15	1	3	55,67	2,05	3,16
16	2	3	55,74	2,07	1,78
17	1	3	ND	ND	ND
18	3	3	62,37	ND	ND
19	1	3	52,24	1,95	2,50
20	3	3	56,33	2,13	2,19
21	2	3	67,58	1,71	2,30
22	3	3	67,40	1,96	2,42
23	1	3	59,38	1,56	1,84
24	2	3	60,32	1,80	3,34
25	1	3	57,76	1,66	2,35
26	3	3	52,88	1,79	1,97
27	2	3	61,70	1,91	2,52
28	1	3	55,95	1,85	2,03
29	2	3	55,95	1,67	2,15
30	3	3	59,05	1,67	2,62
1	1	4	48,97	1,37	1,76
2	3	4	58,16	1,66	2,09
3	2	4	50,35	1,70	1,88
4	3	4	49,86	2,17	2,42
5	1	4	51,38	1,71	2,00
6	2	4	57,21	1,58	1,77
7	1	4	53,57	1,27	2,27
8	2	4	54,42	1,28	2,33
9	3	4	59,32	1,81	1,94
10	2	4	ND	1,56	2,50

11	1	4	56,17	1,83	2,41
12	3	4	52,39	1,39	2,00
13	2	4	51,30	0,89	2,27
14	3	4	51,96	1,22	2,17
15	1	4	53,03	1,68	2,32
16	2	4	62,26	1,54	1,84
17	1	4	53,71	1,73	2,33
18	3	4	54,41	1,74	1,99
19	1	4	52,47	1,64	2,86
20	3	4	55,92	2,09	2,78
21	2	4	ND	2,08	2,37
22	3	4	49,67	1,53	2,12
23	1	4	51,07	1,18	1,98
24	2	4	51,28	1,59	1,93
25	1	4	49,68	1,27	1,80
26	3	4	50,21	1,99	2,07
27	2	4	50,78	1,50	2,06
28	1	4	49,20	1,56	2,23
29	2	4	58,28	1,42	2,50
30	3	4	48,40	1,80	2,29

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 - 35 Semanas; Período 2 - 45 Semanas; Período 3 - 55 Semanas; Período 4 - 65 Semanas;
ND – não determinado.

Apêndice 11. Espessura e calcificação da tíbia aos 10 dias de incubação

Box	Trt	Período	Espessura Tíbia, mm	Calcificação Tíbia, %
1	1	1	0,046	0,066
2	3	1	0,047	0,080
3	2	1	0,045	0,087
4	3	1	0,046	0,071
5	1	1	0,045	0,101
6	2	1	0,046	0,090
7	1	1	0,047	0,090
8	2	1	0,047	0,100
9	3	1	0,046	0,093
10	2	1	0,049	0,110
11	1	1	0,049	0,081
12	3	1	0,052	0,101
13	2	1	0,051	0,109
14	3	1	0,050	0,129
15	1	1	0,047	0,114
16	2	1	0,051	0,069
17	1	1	0,052	0,094
18	3	1	0,049	0,089
19	1	1	0,046	0,063
20	3	1	0,048	0,093
21	2	1	0,042	0,073
22	3	1	0,046	0,076
23	1	1	0,050	0,096
24	2	1	0,052	0,114
25	1	1	0,046	0,072
26	3	1	0,043	0,063
27	2	1	0,053	0,108
28	1	1	0,048	0,126
29	2	1	0,047	0,121
30	3	1	0,053	0,154
1	1	2	0,053	0,051
2	3	2	0,049	0,068
3	2	2	0,049	0,075
4	3	2	0,059	0,109
5	1	2	0,055	0,106
6	2	2	0,056	0,121
7	1	2	0,052	0,106
8	2	2	0,053	0,092
9	3	2	ND	ND
10	2	2	ND	ND
11	1	2	ND	ND
12	3	2	0,046	0,075
13	2	2	0,049	0,087
14	3	2	0,052	0,092
15	1	2	0,046	0,045
16	2	2	0,051	0,057
17	1	2	0,056	0,073
18	3	2	ND	ND
19	1	2	0,060	0,113

20	3	2	ND	ND
21	2	2	0,046	0,070
22	3	2	ND	ND
23	1	2	0,053	0,076
24	2	2	ND	ND
25	1	2	0,050	0,065
26	3	2	0,047	0,086
27	2	2	ND	ND
28	1	2	0,045	0,063
29	2	2	ND	ND
30	3	2	0,050	0,073
1	1	3	ND	0,102
2	3	3	0,059	0,111
3	2	3	0,058	0,094
4	3	3	0,056	0,082
5	1	3	0,052	0,065
6	2	3	0,053	0,074
7	1	3	0,053	0,079
8	2	3	0,060	0,083
9	3	3	0,056	0,098
10	2	3	0,050	0,066
11	1	3	0,064	0,095
12	3	3	0,058	0,061
13	2	3	0,051	0,057
14	3	3	0,047	0,038
15	1	3	0,051	0,021
16	2	3	0,051	0,042
17	1	3	0,048	0,027
18	3	3	0,047	0,024
19	1	3	ND	ND
20	3	3	0,055	0,074
21	2	3	0,049	0,055
22	3	3	ND	ND
23	1	3	0,052	0,081
24	2	3	ND	ND
25	1	3	0,049	0,036
26	3	3	0,048	0,049
27	2	3	0,042	0,046
28	1	3	0,049	0,081
29	2	3	0,052	0,032
30	3	3	0,048	0,037
1	2	3	0,052	0,082
2	3	3	0,049	0,099
3	2	4	0,054	0,109
4	3	4	0,050	0,065
5	1	4	ND	ND
6	2	4	0,056	0,047
15	1	4	ND	ND
19	1	4	0,055	0,050
22	3	4	0,047	0,000
27	2	4	0,054	0,046

28	1	4	0,049	0,099
29	2	4	0,054	0,109
30	3	4	0,050	0,065

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 - 30 Semanas; Período 2 - 40 Semanas; Período 3 - 50 Semanas; Período 4 - 60 Semanas;
ND – não determinado.

Apêndice 12. Espessura e calcificação da tíbia e fêmur de embriões aos 14 dias de incubação

Box	Trt	Período	Espessura Tíbia, mm	Calcificação Tíbia, %	Espessura Fêmur, mm	Calcificação Fêmur, %
1	1	1	0,121	70,052	0,135	71,277
2	3	1	0,125	73,157	0,145	69,287
3	2	1	0,151	72,308	0,142	67,466
4	3	1	0,138	72,068	0,137	69,006
5	1	1	0,130	71,535	0,123	68,306
6	2	1	0,123	70,332	0,141	68,270
7	1	1	0,150	71,818	0,130	68,678
8	2	1	0,143	72,623	0,136	69,481
9	3	1	0,142	71,705	0,133	69,204
10	2	1	0,139	69,897	0,129	68,577
11	1	1	0,138	70,211	0,136	67,041
12	3	1	0,141	72,595	0,145	73,231
13	2	1	0,122	69,727	0,127	65,173
14	3	1	0,124	69,454	0,128	65,789
15	1	1	0,121	69,081	0,119	64,633
16	2	1	0,120	72,170	0,127	66,382
17	1	1	0,127	66,132	0,114	63,843
18	3	1	0,117	69,323	0,123	66,275
19	1	1	0,120	68,115	0,120	65,583
20	3	1	0,143	68,622	0,141	65,238
21	2	1	0,113	71,648	0,112	71,552
22	3	1	0,123	71,049	0,112	66,829
23	1	1	0,122	70,941	0,131	67,992
24	2	1	0,134	69,546	0,122	65,290
25	1	1	0,131	71,582	0,136	68,156
26	3	1	0,144	70,121	0,127	68,920
27	2	1	0,121	69,460	0,136	65,014
28	1	1	0,125	68,374	0,127	66,190
29	2	1	0,114	69,943	0,114	67,087
30	3	1	0,135	69,986	0,119	67,827
1	1	2	0,148	67,022	0,136	70,587
2	3	2	0,138	71,597	0,135	72,571
3	2	2	0,161	72,584	0,146	68,987
4	3	2	0,154	72,448	0,136	68,434
5	1	2	0,145	71,424	0,137	69,989
6	2	2	0,148	70,594	0,121	68,116
7	1	2	0,144	68,929	0,135	66,473
8	2	2	0,151	69,731	0,134	68,733
9	3	2	0,144	68,171	0,130	69,040
10	2	2	0,157	71,212	0,145	69,246
11	1	2	0,146	71,544	0,124	69,160
12	3	2	0,145	68,442	0,126	69,087
13	2	2	0,144	68,794	0,132	69,411
14	3	2	0,155	69,597	0,129	71,184
15	1	2	0,148	68,130	0,134	68,519
16	2	2	0,132	67,516	0,126	66,419
17	1	2	0,154	67,992	0,123	64,160

18	3	2	0,137	68,762	0,120	68,975
19	1	2	0,145	69,759	0,130	66,737
20	3	2	0,143	67,041	0,126	66,568
21	2	2	0,139	69,831	0,125	64,558
22	3	2	0,142	66,530	0,118	68,646
23	1	2	0,156	65,643	0,131	66,568
24	2	2	0,131	69,948	0,124	62,289
25	1	2	0,149	67,324	0,132	69,021
26	3	2	0,149	69,268	0,147	68,950
27	2	2	0,137	68,080	0,121	64,854
28	1	2	0,134	62,839	0,111	62,491
29	2	2	0,143	68,025	0,120	68,972
30	3	2	0,139	66,529	0,135	66,939
1	1	3	0,138	72,857	0,122	71,910
2	3	3	0,156	73,750	0,132	72,920
3	2	3	0,156	73,117	0,136	74,491
4	3	3	0,181	73,311	0,149	68,402
5	1	3	0,163	71,302	0,146	65,954
6	2	3	0,163	73,494	0,135	72,992
7	1	3	0,154	73,424	0,140	72,043
8	2	3	0,141	71,757	0,133	70,065
9	3	3	0,156	72,244	0,139	69,595
10	2	3	0,146	74,712	0,139	69,516
11	1	3	0,148	73,252	0,134	70,899
12	3	3	0,144	72,492	0,132	69,435
13	2	3	0,140	71,390	0,127	68,951
14	3	3	0,142	72,781	0,133	69,991
15	1	3	0,139	71,985	0,125	68,135
16	2	3	0,149	70,019	0,135	66,731
17	1	3	0,158	69,723	0,124	65,468
18	3	3	0,145	71,856	0,121	74,650
19	1	3	0,154	70,832	0,126	69,539
20	3	3	0,146	69,607	0,127	69,399
21	2	3	0,149	70,803	0,117	68,969
22	3	3	0,155	71,204	0,128	67,642
23	1	3	0,158	71,969	0,133	67,629
24	2	3	0,148	71,586	0,129	68,720
25	1	3	0,149	70,269	0,122	69,279
26	3	3	0,142	72,402	0,125	68,319
27	2	3	0,148	71,301	0,120	64,415
28	1	3	0,151	70,278	0,133	65,883
29	2	3	0,141	69,969	0,122	65,192
30	3	3	0,147	70,697	0,130	68,930
31	2	3	0,141	68,800	0,125	65,570
32	3	3	0,148	69,932	0,139	66,763
1	1	4	0,174	67,104	0,149	68,063
2	3	4	0,175	66,156	0,137	67,821
3	2	4	0,171	71,172	0,137	69,971
4	3	4	0,166	69,501	0,148	69,148
5	1	4	0,165	68,531	0,133	68,862
6	2	4	0,168	70,127	0,140	69,003

7	1	4	0,157	66,565	0,142	68,902
8	2	4	0,163	67,700	0,132	69,429
9	3	4	0,158	67,596	0,135	69,063
10	2	4	0,168	69,314	0,133	68,901
11	1	4	0,172	69,756	0,146	68,760
12	3	4	0,154	69,059	0,135	72,348
13	2	4	0,170	70,893	0,147	68,918
14	3	4	0,163	66,155	0,130	66,217
15	1	4	0,154	69,435	0,126	68,852
16	2	4	0,148	65,387	0,135	65,849
17	1	4	0,150	66,081	0,139	67,275
18	3	4	0,172	69,362	0,144	70,200
19	1	4	0,146	65,932	0,118	67,545
20	3	4	0,160	71,654	0,132	68,930
21	2	4	0,153	67,040	0,132	66,386
22	3	4	0,166	66,827	0,141	68,551
23	1	4	0,150	65,101	0,134	68,837
24	2	4	0,160	67,248	0,132	68,680
25	1	4	0,153	67,026	0,134	68,648
26	3	4	0,153	67,923	0,123	68,898
27	2	4	0,144	67,949	0,124	61,513
28	1	4	0,153	66,750	0,133	65,441
29	2	4	0,144	71,848	0,127	69,749
30	3	4	0,151	66,590	0,130	65,552

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 - 30 Semanas; Período 2 - 40 Semanas; Período 3 - 50 Semanas; Período 4 - 60 Semanas.

Apêndice 13. Espessura e calcificação da tíbia e fêmur de embriões aos 18 dias de incubação

Box	Trt	Período	Espessura Tíbia, mm	Calcificação Tíbia, %	Espessura Fêmur, mm	Calcificação Fêmur, %
1	1	1	0,187	77,397	0,202	74,397
2	3	1	0,216	82,430	0,224	75,447
3	2	1	0,198	78,693	0,204	79,740
4	3	1	0,187	75,836	0,165	76,945
5	1	1	0,203	77,908	0,212	75,904
6	2	1	0,194	79,309	0,192	75,501
7	1	1	0,173	76,348	0,207	77,985
8	2	1	0,202	77,692	0,224	72,868
9	3	1	0,217	77,693	0,188	80,231
10	2	1	0,236	76,676	0,261	76,206
11	1	1	0,190	77,614	0,197	75,807
12	3	1	0,190	77,532	0,193	77,310
13	2	1	0,201	78,025	0,184	76,957
14	3	1	0,214	76,983	0,205	76,045
15	1	1	0,198	77,136	0,193	76,819
16	2	1	0,210	74,464	0,190	74,831
17	1	1	0,198	75,707	0,201	73,321
18	3	1	0,210	77,172	0,198	76,912
19	1	1	0,189	75,749	0,182	73,229
20	3	1	0,200	76,537	0,227	74,624
21	2	1	0,204	76,502	0,193	74,593
22	3	1	0,199	77,734	0,197	76,225
23	1	1	0,192	76,692	0,199	76,241
24	2	1	0,196	77,460	0,211	72,138
25	1	1	0,202	75,391	0,201	76,128
26	3	1	0,205	77,484	0,189	74,836
27	2	1	0,191	74,884	0,207	71,866
28	1	1	0,184	76,978	0,194	74,130
29	2	1	0,203	75,599	0,204	76,904
30	3	1	0,196	79,519	0,180	76,150
1	1	2	0,195	73,967	0,196	76,957
2	3	2	0,204	78,877	0,216	77,665
3	2	2	0,218	79,458	0,235	78,092
4	3	2	0,229	80,617	0,242	76,008
5	1	2	0,209	74,133	0,214	74,998
6	2	2	0,205	76,741	0,221	76,119
7	1	2	0,206	77,659	0,214	76,071
8	2	2	0,187	77,551	0,209	76,757
9	3	2	0,204	79,540	0,219	75,511
10	2	2	0,208	76,816	0,216	75,174
11	1	2	0,203	75,945	0,211	74,963
12	3	2	0,202	76,073	0,230	74,769
13	2	2	0,205	75,644	0,204	75,903
14	3	2	0,202	77,328	0,207	75,862
15	1	2	0,214	76,636	0,211	75,689
16	2	2	0,203	75,178	0,226	74,491
17	1	2	0,200	77,431	0,200	76,470

18	3	2	0,172	78,011	0,210	76,033
19	1	2	0,202	76,811	0,206	76,224
20	3	2	0,201	74,907	0,209	76,200
21	2	2	0,202	75,549	0,203	75,983
22	3	2	0,218	78,744	0,220	77,063
23	1	2	0,214	76,026	0,228	74,004
24	2	2	0,215	75,689	0,215	75,431
25	1	2	0,202	77,453	0,208	77,805
26	3	2	0,208	76,660	0,198	75,435
27	2	2	0,213	77,528	0,207	75,913
28	1	2	0,197	77,421	0,213	75,837
29	2	2	0,213	76,427	0,210	76,514
30	3	2	0,216	78,458	0,201	76,130
1	1	3	0,219	78,470	0,210	76,895
2	3	3	0,212	77,401	0,223	76,555
3	2	3	0,230	77,173	0,215	77,534
4	3	3	0,214	76,856	0,211	76,866
5	1	3	0,212	77,590	0,215	75,058
6	2	3	0,230	78,373	0,225	77,128
7	1	3	0,226	76,723	0,211	74,733
8	2	3	0,215	77,319	0,219	77,144
9	3	3	0,214	77,388	0,219	75,369
10	2	3	0,219	78,009	0,215	76,039
11	1	3	0,210	77,320	0,195	76,585
12	3	3	0,199	76,806	0,208	78,551
13	2	3	0,208	77,378	0,208	78,387
14	3	3	0,199	78,553	0,214	77,785
15	1	3	0,193	76,027	0,209	75,100
16	2	3	0,206	76,852	0,220	77,078
17	1	3	0,198	76,954	0,204	76,661
18	3	3	0,209	77,526	0,218	76,537
19	1	3	0,220	76,538	0,213	75,562
20	3	3	0,205	77,652	0,211	76,952
21	2	3	0,208	77,451	0,212	77,762
22	3	3	0,208	75,865	0,209	77,063
23	1	3	0,207	76,834	0,212	78,047
24	2	3	0,192	77,487	0,203	75,808
25	1	3	0,190	77,527	0,195	75,383
26	3	3	0,200	77,234	0,206	75,765
27	2	3	0,196	77,796	0,202	76,374
28	1	3	0,198	78,456	0,203	76,979
29	2	3	0,199	76,392	0,207	76,297
30	3	3	0,212	76,895	0,197	77,922
31	2	3	0,217	77,694	0,212	76,469
32	3	3	0,212	79,090	0,207	76,872
1	1	4	0,215	75,597	0,212	72,697
2	3	4	0,210	78,176	0,213	74,756
3	2	4	0,229	76,496	0,225	74,325
4	3	4	0,215	78,223	0,209	76,268
5	1	4	0,197	78,323	0,206	76,284
6	2	4	0,215	76,297	0,219	75,051

7	1	4	0,209	73,714	0,214	76,426
8	2	4	0,214	75,443	0,230	74,949
9	3	4	0,219	76,113	0,227	77,291
10	2	4	0,221	76,450	0,233	76,566
11	1	4	0,214	78,251	0,210	77,122
12	3	4	0,217	76,759	0,224	77,144
13	2	4	0,216	76,072	0,225	76,348
14	3	4	0,203	77,129	0,220	77,184
15	1	4	0,201	76,027	0,217	76,015
16	2	4	0,203	77,540	0,219	77,436
17	1	4	0,218	77,601	0,222	77,192
18	3	4	0,225	76,344	0,222	77,209
19	1	4	0,194	77,363	0,214	76,598
20	3	4	0,200	78,715	0,215	79,280
21	2	4	0,196	77,887	0,213	76,094
22	3	4	0,197	80,071	0,217	76,833
23	1	4	0,207	76,451	0,213	76,423
24	2	4	0,221	78,605	0,217	77,851
25	1	4	0,211	75,842	0,219	76,706
26	3	4	0,215	76,941	0,228	76,416
27	2	4	0,211	76,477	0,220	73,711
28	1	4	0,214	77,580	0,225	76,452
29	2	4	0,213	76,899	0,210	76,816
30	3	4	0,218	76,003	0,231	78,854

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 - 30 Semanas; Período 2 - 40 Semanas; Período 3 - 50 Semanas; Período 4 - 60 Semanas.

Apêndice 14. Peso, cinzas e comprimento de tíbia de pintinhos pós-eclosão

Box	Trt	Período	Peso tíbia, g	Cinzas na tíbia, %	Comprimento tíbia, mm
1	1	1	0,078	25,121	31,29
2	3	1	0,089	27,466	31,05
3	2	1	0,079	28,587	32,09
4	3	1	0,077	27,294	30,83
5	1	1	0,084	25,340	31,20
6	2	1	0,074	25,974	30,37
7	1	1	0,082	26,060	31,36
8	2	1	0,081	26,933	31,49
9	3	1	0,074	25,945	31,43
10	2	1	0,078	27,859	31,19
11	1	1	0,079	22,702	30,76
12	3	1	0,077	24,766	31,47
13	2	1	0,071	22,652	30,61
14	3	1	0,082	34,439	30,69
15	1	1	0,084	26,382	31,49
16	2	1	0,074	26,101	31,03
17	1	1	0,078	26,677	31,46
18	3	1	0,079	26,045	31,48
19	1	1	0,066	21,925	29,84
20	3	1	0,089	25,922	30,91
21	2	1	0,074	27,891	31,07
22	3	1	0,076	27,732	30,32
23	1	1	0,076	31,286	29,90
24	2	1	0,078	31,371	30,61
25	1	1	0,074	30,074	30,51
26	3	1	0,089	26,101	31,40
27	2	1	0,075	31,101	30,92
28	1	1	0,078	28,253	30,05
29	2	1	0,079	29,994	30,26
30	3	1	0,080	28,866	30,58
1	1	2	0,0900	26,789	32,46
2	3	2	0,0970	29,160	31,73
3	2	2	0,1015	30,611	31,76
4	3	2	0,0925	29,563	31,21
5	1	2	0,0970	29,264	32,29
6	2	2	0,0900	30,838	32,10
7	1	2	0,0970	26,807	31,77
8	2	2	0,0890	26,781	32,51
9	3	2	0,0850	29,703	32,38
10	2	2	0,0990	29,921	31,42
11	1	2	0,0890	32,542	31,36
12	3	2	0,0855	27,625	31,87
13	2	2	0,0820	28,230	31,21
14	3	2	0,0940	28,444	32,25
15	1	2	0,0940	29,792	32,29
16	2	2	0,0895	29,336	31,53
17	1	2	0,0880	29,611	31,20
18	3	2	0,0915	26,263	32,88
19	1	2	0,0875	28,735	31,19

20	3	2	0,0860	30,824	31,78
21	2	2	0,0930	30,775	31,27
22	3	2	0,0815	30,750	30,61
23	1	2	0,0855	29,048	31,39
24	2	2	0,0890	29,046	32,81
25	1	2	0,0880	29,100	30,79
26	3	2	0,0890	30,189	31,55
27	2	2	0,0890	30,288	31,29
28	1	2	0,0815	27,770	31,76
29	2	2	0,0915	27,484	31,12
30	3	2	0,0935	27,475	31,18
1	1	3	0,1080	23,386	32,91
2	3	3	0,1035	27,391	32,53
3	2	3	0,0910	31,234	32,62
4	3	3	0,0915	29,085	32,70
5	1	3	0,0900	24,663	33,60
6	2	3	0,0940	25,566	32,81
7	1	3	0,0920	28,757	31,33
8	2	3	0,0990	22,918	32,62
9	3	3	0,0925	28,955	31,78
10	2	3	0,0970	30,251	32,38
11	1	3	0,0890	30,775	31,93
12	3	3	0,1025	28,524	33,05
13	2	3	0,0975	27,935	32,16
14	3	3	0,0970	29,518	32,68
15	1	3	0,0900	25,121	32,06
16	2	3	0,0865	25,505	32,15
17	1	3	0,1020	26,344	32,14
18	3	3	0,1005	26,097	32,55
19	1	3	0,0845	23,959	30,87
20	3	3	0,0845	29,166	31,51
21	2	3	0,0950	29,882	31,73
22	3	3	0,0975	25,848	30,82
23	1	3	0,0890	27,965	32,02
24	2	3	0,0995	28,886	33,03
25	1	3	0,0970	29,119	32,10
26	3	3	0,1085	29,328	32,91
27	2	3	0,1050	26,386	31,38
28	1	3	0,0885	30,314	32,30
29	2	3	0,0875	28,880	31,41
30	3	3	0,0990	28,846	31,87

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 - 30 Semanas; Período 2 - 40 Semanas; Período 3 - 50 Semanas.

Apêndice 15. Avaliações histológicas e morfometria da tíbia de pintinhos ao nascimento

Box	Trt	Período	Espessura disco de crescimento, mm	Área do disco de crescimento, mm2	Momento de inércia, mm4	Área da tábua óssea, mm²
1	1	1	0,606	5,662	1,138	2,922
2	3	1	0,623	5,627	1,141	2,629
3	2	1	0,656	6,317	0,993	2,452
4	3	1	0,649	6,243	1,221	2,556
5	1	1	0,610	5,990	0,922	2,555
6	2	1	0,662	6,178	1,023	2,599
7	1	1	0,632	5,651	0,888	2,301
8	2	1	0,681	5,947	0,933	2,417
9	3	1	0,659	5,890	1,193	2,584
10	2	1	0,588	5,721	1,008	2,299
11	1	1	0,607	5,866	0,802	2,232
12	3	1	0,648	6,101	1,143	2,533
13	2	1	0,640	6,120	0,981	2,664
14	3	1	0,664	5,790	1,184	2,471
15	1	1	0,636	5,806	0,807	2,310
16	2	1	0,649	6,063	0,878	2,347
17	1	1	0,646	5,760	0,935	2,339
18	3	1	0,654	5,673	1,011	2,292
19	1	1	0,656	6,065	0,902	2,411
20	3	1	0,593	6,235	1,231	2,499
21	2	1	0,619	5,658	1,152	2,471
22	3	1	0,646	6,140	1,164	2,480
23	1	1	0,629	6,469	1,073	2,519
24	2	1	0,628	5,701	1,153	2,583
25	1	1	0,642	5,850	1,018	2,561
26	3	1	0,619	5,890	0,832	2,068
27	2	1	0,622	5,930	1,256	2,542
28	1	1	0,673	6,014	0,973	2,304
29	2	1	0,604	5,790	1,075	2,429
30	3	1	0,626	5,663	1,124	2,457
1	1	2	0,576	6,132	1,185	2,567
2	3	2	0,622	6,489	1,117	2,728
3	2	2	0,642	6,250	1,253	2,575
4	3	2	0,691	6,245	1,293	2,625
5	1	2	0,594	6,112	0,939	2,285
6	2	2	0,630	6,230	1,184	2,475
7	1	2	0,616	6,254	1,026	2,349
8	2	2	0,623	6,444	1,178	2,472
9	3	2	0,655	6,238	1,044	2,502
10	2	2	0,667	6,403	1,206	2,508
11	1	2	0,636	5,898	1,301	2,708
12	3	2	0,620	6,456	1,127	2,489
13	2	2	0,644	6,070	1,060	2,346
14	3	2	0,614	6,287	1,121	2,299
15	1	2	0,606	6,443	0,968	2,611
16	2	2	0,635	6,314	1,039	2,348
17	1	2	0,599	6,223	1,034	2,458

18	3	2	0,590	6,124	1,188	2,560
19	1	2	0,674	6,456	1,042	2,527
20	3	2	0,554	6,562	1,285	2,487
21	2	2	0,636	5,990	1,162	2,616
22	3	2	0,604	5,918	1,290	2,654
23	1	2	0,600	5,940	0,953	2,291
24	2	2	0,600	6,330	1,104	2,689
25	1	2	0,656	6,175	1,000	2,400
26	3	2	0,603	6,322	1,206	2,628
27	2	2	0,637	6,310	1,178	2,417
28	1	2	0,610	5,825	0,983	2,394
29	2	2	0,553	6,630	1,151	2,494
30	3	2	0,650	5,905	1,206	2,628
1	1	3	0,589	6,412	0,992	2,365
2	3	3	0,624	6,687	1,179	2,665
3	2	3	0,639	6,342	0,938	2,301
4	3	3	0,623	6,360	1,204	2,664
5	1	3	0,585	6,292	0,914	2,393
6	2	3	0,610	6,238	0,899	2,255
7	1	3	0,659	6,046	1,053	2,513
8	2	3	0,621	6,677	1,192	2,557
9	3	3	0,631	6,614	1,101	2,443
10	2	3	0,627	6,824	1,296	2,669
11	1	3	0,639	5,898	1,259	2,639
12	3	3	0,610	6,152	1,163	2,508
13	2	3	0,621	6,302	1,136	2,600
14	3	3	0,597	6,184	1,065	2,434
15	1	3	0,641	5,850	1,360	2,499
16	2	3	0,670	6,070	1,205	2,562
17	1	3	0,642	5,852	1,371	2,623
18	3	3	0,583	6,330	1,153	2,557
19	1	3	0,588	5,898	1,176	2,616
20	3	3	0,610	6,030	1,071	2,360
21	2	3	0,630	6,313	1,233	2,595
22	3	3	0,671	6,905	0,970	2,196
23	1	3	0,574	6,610	0,893	2,347
24	2	3	0,650	6,090	1,076	2,388
25	1	3	0,602	6,430	1,054	2,500
26	3	3	0,657	6,757	1,156	2,545
27	2	3	0,592	6,120	1,210	2,590
28	1	3	0,669	6,286	1,056	2,498
29	2	3	0,556	6,095	1,210	2,590
30	3	3	0,625	6,314	1,156	2,545

Tratamentos: T1 - CONTROL; T2 - ISO; T3 - ON TOP;
Período 1 - 30 Semanas; Período 2 - 40 Semanas; Período 3 - 50 Semanas.

Apêndice 16.Modelo geral do utilizado para as análises estatísticas pelo pacote estatístico SAS, 2010

```
proc import
datafile="C:\dados.xls"
out=work.TEMP1
DBMS=EXCEL97 replace;
run;

proc contents;
run;

proc mixed;
 class BOX TRT Tempo;
 model variavel = TRT tempo TRT*Tempo;
repeated tempo / TYPE = cs sub=Box(TRT) R RCORR;
run;

proc mixed;
 class BOX TRT Tempo;
 model variavel = TRT tempo TRT*Tempo;
repeated tempo / TYPE = AR(1) sub=Box(TRT) R RCORR;
run;

proc mixed;
 class BOX TRT Tempo;
 model variavel = TRT tempo TRT*Tempo;
repeated tempo / TYPE = UN sub=Box (TRT) R RCORR;
run;

proc mixed;
 class BOX TRT Tempo;
 model variavel = TRT tempo TRT*Tempo;
repeated tempo / TYPE = HF sub=Box(TRT) R RCORR;
run;

proc mixed;
 class BOX TRT Tempo;
 model variavel = TRT tempo TRT*Tempo;
repeated tempo / TYPE = toep sub=Box(TRT) R RCORR;
run;

proc mixed;
 class BOX TRT Tempo;
 model variavel = TRT tempo TRT*Tempo;
repeated tempo / TYPE = ARMA(1,1) sub=Box(TRT) R RCORR;
run;

  proc import
datafile="C:\dados.xls"
out=work.TEMP1
DBMS=EXCEL97 replace;
run;

proc contents;
run;

proc print;
proc univariate plot normal; by Tempo;
var variavel;
run;
proc mixed;
 class BOX TRT Tempo;
 model variavel = TRT tempo TRT*Tempo;
  repeated Tempo / TYPE = AR (1) sub=Box(TRT) R RCORR;
  lsmeans TRT Tempo TRT*Tempo/ PDIFF adjust=TUKEY;
  run;
```

VITA

André Favero, primogênito de Alceu Favero e Elizete Bortoli Favero, casado com Viviane Grutzmacher Azeredo Favero, nasceu em Garibaldi, RS, em 06 de março de 1984. Cursou o ensino fundamental no colégio Santa Inês em Garibaldi, RS. Concluiu o ensino médio no Centro de Estudos Técnicos da UCS (CETEC) em Bento Gonçalves, RS. Em 2002 ingressou no Curso de Zootecnia da Universidade Federal de Santa Maria, RS, obtendo o Grau de Zootecnista em março de 2007. Iniciou, em março de 2007, o curso de mestrado na área de Produção Animal, no Programa de Pós-Graduação em Ciência Veterinárias da Universidade Federal do Paraná, Curitiba, PR. No ano de 2009, prosseguiu em suas pesquisas na área de Produção Animal quando iniciou seu doutorado no Programa de Pós-Graduação em Zootecnia pela Universidade Federal do Rio Grande do Sul, Porto Alegre, RS. Durante o ano de 2012, realizou pesquisas durante 8 meses na Universidade de Purdue, EUA, através do programa doutorado-sanduíche da CAPES e no momento esta finalizando seu doutorado pela Universidade Federal do Rio Grande do Sul em Porto Alegre, RS.