PÂMELA LACOMBE RETES

PROTEÍNA BRUTA NA DIETA E CARACTERÍSTICAS REPRODUTIVAS DE CODORNAS JAPONESAS (*Coturnix coturnix japonica*) MACHOS E FÊMEAS

LAVRAS – MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciências Veterinárias, área de concentração Ciências Veterinárias, para a obtenção do título de Doutor.

Orientador
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APROVADA em 26 de outubro de 2018
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RESUMO

Objetivou-se avaliar diferentes níveis de proteína bruta (PB) dietéticas em codornas japonesas (*Coturnix coturnix japonica*) machos e fêmeas. Dois experimentos foram realizados, um para avaliar variação da PB nas dietas de crescimento e produção (experimento 1) e outro para avaliar variação de PB somente na fase de crescimento (experimento 2). Inicialmente, 300 machos e 640 fêmeas de um dia de idade foram separadamente alojadas em 30 gaiolas contendo 10 machos cada (seis gaiolas por tratamento) e 10 gaiolas com 36 fêmeas cada (duas gaiolas por tratamento). Adicionalmente, oito gaiolas extras com 35 aves cada foram utilizadas para alojar 280 fêmeas para o teste de fertilidade dos machos. Cinco níveis de PB dietética foram utilizados (18, 20, 22, 24 e 26%) até 35 dias de idade. A partir dessa idade, as aves foram redistribuídas em 70 gaiolas contendo nove fêmeas e três machos cada. Quarenta gaiolas (oito por tratamento) continuaram recebendo as rações com diferentes níveis de PB, porém reduzidas em quatro unidades percentuais (14, 16, 18, 20 e 22%) em relação à fase anterior. As outras 30 gaiolas (seis para cada tratamento) passaram a receber apenas ração com o nível de PB recomendado para essa fase (18%). Durante todo o experimento, utilizou-se o delineamento inteiramente casualizado. A cada três dias, 12 aves de cada parcela foram individualmente pesadas até os 60 dias de idade para a determinação da curva de crescimento. Nos machos do experimento 1 houve aumento linear ($P<0,01$) da velocidade de crescimento com o aumento da PB. Maior peso à maturidade ($P<0,05$) foi obtido com 18%. Não houve efeito ($P<0,05$) da PB sobre as características histológicas dos testículos e fisiológicas do sêmen, nem sobre a fertilidade. Nas fêmeas, 24 e 26% de PB reduziram ($P<0,01$) a velocidade de crescimento e aumentaram ($P<0,05$) a idade de máximo ganho de peso. O teor de 24% aumentou ($P<0,01$) o peso à maturidade, enquanto 26% reduziu ($P<0,01$) a idade ao primeiro ovo e aumentou a massa de ovos. Aos 47 dias maiores intensidades de postura ($P<0,05$) foram obtidos com 24 e 26% de PB. Nos machos do experimento 2, a taxa de crescimento aumentou linearmente ($P<0,01$). Aos 36 dias, maior desenvolvimento histológico dos testículos foi observado ($P<0,05$), entretanto, não houve efeito ($P>0,05$) nas características do sêmen ou na fertilidade. Nas fêmeas aos 36 dias a PB estimulou ($P<0,05$) o desenvolvimento anatômico dos ovários, porém, o mesmo não foi observado ($P<0,05$) nas idades posteriores. Aos 48 dias, aumento linear na intensidade de postura foi observado ($P<0,01$) com o aumento da PB dietética. Aumento linear ($P<0,05$) no peso dos ovos foi observado até o final do experimento. Não houve efeito ($P>0,05$) nas características internas do ovo. Conclui-se que os níveis de PB dietética influenciam o desenvolvimento corporal de codornas machos e fêmeas, porém, afetam a qualidade reprodutiva apenas nas fêmeas. Para maior produção até o pico da postura e maior peso dos ovos posterior à essa fase, recomenda-se o uso de 26% de PB em dietas de crescimento e 22% em dietas de produção.

Palavras-chave: Ave, coturnicultura, curva de crescimento, produção de ovos, fertilidade, qualidade do sêmen

ABSTRACT

The aim was to evaluate the effects of different dietary crude protein (CP) levels in male and female Japanese quails. Two experiments were carried out: one to evaluate CP variation according to growth and production diets (experiment 1), and another to evaluate CP variation only in the growth phase (experiment 2). Initially, 300 one-day-old males and 640 one-day-old females were housed in 30 cages containing 10 males each (6 cages per treatment) and 10 cages with 36 females each (2 cages per treatment). In addition, 8 extra cages containing 35 birds each were used to house 280 additional females for the fertility test of the males. Five levels of dietary CP were used (18, 20, 22, 24, and 26%) up to 35 days of age. From that age, the birds were redistributed in 70 cages containing nine females and three males each. Birds from forty cages (eight per treatment) continued to be fed diets with different CP levels, but reduced by four percentage units (14, 16, 18, 20, and 22%) relative to the previous phase. The other 30 cages (6 for each treatment) received only feed with the CP levels recommended for this phase (18%). Throughout the experiment, a completely randomized design was used. Every 3 days until 60 days of age, 12 birds from each plot were individually weighed to determine the growth curve. The males from experiment 1 demonstrated a linear increase ($P<0.01$) in growth rate with increasing CP. A highest weight at maturity ($P<0.05$) was obtained with 18% PB. There was no effect ($P<0.05$) of CP on the histological characteristics of the testes or the physiological characteristics of the semen, or on fertility. In females, 24 and 26% CP reduced ($P<0.01$) the growth rate and increased ($P<0.05$) the age of maximum weight gain. The level of 24% CP resulted in higher ($P<0.01$) weight at maturity, whereas 26% showed lower ($P<0.01$) age at the first egg and higher egg mass compared to other CP levels. At 47 days, the highest posture intensity ($P<0.05$) was obtained with 24 and 26% CP. The growth rate of the males from experiment 2 and the histological development of the testes increased linearly ($P<0.01$) with increase of dietary CP. However, there was no effect ($P>0.05$) on semen characteristics or fertility. In females at 36 days, CP stimulated ($P<0.05$) the anatomical development of the ovaries, however, this effect was not observed ($P<0.05$) at later ages. At 48 days, a linear increase in posture intensity was observed ($P<0.01$) with increasing dietary CP. A linear increase ($P<0.05$) in egg weight was observed until the end of the experiment. There was no effect ($P>0.05$) on the internal characteristics of the egg. It is concluded that dietary CP levels influence the body development of males and females but only affect the reproductive quality of females. For a higher production up to the peak of posture and a higher egg weight after this phase, it is recommended to use 26% CP in growth diets and 22% in production diets.

Keywords: Birds, egg produtcion, fertility, growth curve, quail production, semen

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

A coturnicultura começou a ser explorada no Brasil em meados de 1950. A partir de então, houve crescimento expressivo ao longo dos anos. Em 2014, o efetivo nacional de codornas atingiu valores históricos, chegando próximo a 20 milhões de cabeças. No entanto, a perda do poder aquisitivo da população nos anos seguintes resultou na queda da demanda por ovos, principal produto oriundo da coturnicultura. Devido a isso, a produção de ovos, que chegou próximo à 400 milhões de dúzias em 2014, em 2016 foi de apenas 273 milhões de dúzias (IBGE, 2016). Entrentanto, apesar da redução nos índices produtivos, a coturnicultura ainda é uma atividade relevante para a agropecuária brasileira. Sendo assim, estratégias que visam não só o retorno do crescimento, mas também aumente a eficiência do sistema produtivo são importantes.

No que tange às características produtivas em um plantel de matrizes de codornas, poucas informações são encontradas na literatura. Nesse importante segmento da coturnicultura, a fertilidade é uma das características econômicas mais importantes, sendo influenciada por inúmeros fatores, dentre eles, a nutrição. De todos os nutrientes presentes em uma dieta, a proteína bruta (PB) merece destaque, sendo considerada como um dos fatores que mais influenciam os índices reprodutivos de um plantel. Tanto a falta quanto o excesso de aminoácidos podem prejudicar as funções fisiológicas dos animais e, consequentemente, afetar a qualidade reprodutiva dos mesmos.

Na literatura, ainda há grande divergência quanto às reais necessidades nutricionais para codornas japonesas. Murakami et al. (1993) recomendam dietas com 20% de PB na fase de crescimento (1 a 42 dias de idade). Soares et al. (2003) sugerem 23%. Já o NRC (1994) recomenda 24% e Leeson e Summers (2009) 26% de PB. Silva e Costa (2009) sugerem 25% de PB até os 21 dias de idade e 22% até os 42 dias. Rostagno et al. (2011) recomendaram 22% de 1 a 35 dias, porém, em sua versão atualizada, recomendam 24% (1 a 14 dias) e 23% (15 a 35 dias) de PB (ROSTAGNO et al., 2017). Ghazaghi et al. (2012) e Richard et al. (2016) estimaram que 25% e 26% de PB, respectivamente, garantem maior desempenho. Dessa forma, é possível inferir que são necessários mais estudos para elucidar o melhor nível proteico para o máximo desempenho das aves.

Apesar dos inúmeros estudos avaliando níveis de PB na dieta de codornas japonesas, a maioria é baseada no desempenho produtivo de fêmeas. Tendo em vista que a maioria dos

problemas reprodutivos do plantel de matrizes está relacionada à fatores ligados aos machos, já que sua proporção em relação às fêmeas é menor, o conhecimento das reais necessidades nutricionais dessa categoria animal pode melhorar a fertilidade do plantel. Devido às diferenças fisiológicas e metabólicas entre machos e fêmeas, a criação separada por sexo poderia melhorar o desempenho das matrizes. Porém, informações acerca das reais necessidades dos machos de codornas japonesas são ainda escassas na literatura. Dessa forma, objetivou-se avaliar a influência de diferentes níveis de proteína sobre as características reprodutivas de codornas japonesas fêmeas e machos.

2 REVISÃO DE LITERATURA

2.1 Proteína bruta na dieta de codornas japonesas

As proteínas desempenham papéis fundamentais no organismo das aves. Além de serem os principais componentes musculares, representando mais de 20% do peso do músculo na matéria natural, também participam como componentes estruturais ao formarem as fibras colágenas, queratina e estarem presentes na membrana celular. Atuam ainda como proteínas de ligação, receptores, hormônios, enzimas, anticorpos, transportadores, entre outros (SCANES et al., 2014). Entretanto, a manutenção dessas proteínas corporais depende das proteínas dietéticas. Portanto, o fornecimento adequado de proteína na dieta é essencial para permitir o correto funcionamento fisiológico e metabólico das proteínas presentes no organismo.

A proteína dietética digerida libera na corrente sanguínea, prevalentemente, aminoácidos livres. A síntese proteica no organismo é dependente desse *pool* de aminoácidos no plasma (SCANES et al., 2014). Para codornas em crescimento, assim como para outras aves, o aporte de aminoácidos é primeiramente utilizado para atender as exigências de mantença antes que seja destinado às necessidades de crescimento (WEI et al., 2011). Dessa forma, as maiores taxas de síntese de proteína muscular são observadas quando a PB da dieta atende as exigências de mantença e crescimento. As deficiências nutricionais normalmente reduzem a taxa de síntese proteica muscular. Quando mais rigorosas, tais deficiências podem até levar ao catabolismo muscular (SCANES et al., 2014). Portanto, baixos níveis de proteína dietética durante as fases de crescimento de codornas podem limitar o desenvolvimento

corporal devido ao baixo aporte de aminoácidos no plasma (RENEMA et al., 1999; LIMA et al., 2016).

Kirkpinar e Oguz (1995) observaram taxas de crescimento mais rápidas quando codornas japonesas foram alimentadas com maiores níveis de PB na fase de cria e recria. Em seu estudo, esses autores e concluíram que 24% de proteína pode não ser suficiente para altas taxas de crescimento já que codornas alimentadas com 30% de proteína encontravam-se mais pesadas aos 35 dias. Mosaad e Iben (2009), aumentando os níveis de proteína de 21% para 27% na fase de cria e recria, e Alagawany et al. (2014) que aumentaram de 20% para 22% também nessa fase, observaram maior peso corporal aos 21 e 42 dias, respectivamente, nos maiores níveis de PB testados. Portanto, o aumento dos níveis de PB nesses trabalhos pode ter suprido deficiências de aminoácidos que estavam limitando a síntese proteica.

O adequado desenvolvimento corporal das codornas na fase de cria e recria é de grande importância para as características reprodutivas das aves, uma vez que o peso corporal tem correlação direta com órgãos reprodutivos como ovário e oviduto nas fêmeas e testículos nos machos (FONTANA; WEAVER; KREY, 1990; LILBURN; STEIGNER; NESTOR, 1992; RENEMA et al., 1999). Sendo assim, dietas com níveis adequados de proteínas são essenciais para permitir que as aves expressem todo o seu potencial genético de produtividade (BAIÃO; LÚCIO, 2005).

Por outro lado, a utilização de níveis elevados de PB resulta em um aporte de aminoácidos mais elevado do que o necessário (ALAGAWANY et al., 2014). Karaalp et al. (2009) observaram que os aminoácidos glicina + serina, histidina, cistina, triptofano e arginina encontravam-se em excesso nas dietas à base de milho e farelo de soja formuladas para conter 24% de PB, como recomendado pelo NRC (1994). Esse excesso de aminoácidos pode prover prejuízos para as aves que aumentam o gasto energético para a excreção do nitrogênio excedente e, consequentemente, reduzem o desempenho (ALAGAWANY et al., 2014). Sendo assim, a manipulação dietética pode aumentar a eficiência de utilização dos aminoácidos pelo organismo.

O aumento da eficiência de utilização de aminoácidos pelo organismo está diretamente relacionado à diminuição do conteúdo de nitrogênio das excretas e redução dos problemas respiratórios das aves, uma das principais preocupações da indústria avícola (NOVAK; YAKOUT; SCHEIDELER, 2006; ALAGAWANY et al., 2014). Além disso, sabe-se que a alimentação representa mais de 70% dos custos totais de produção. Sendo assim, estratégias como a redução dos níveis de PB na dieta têm sido utilizadas para a formulação de rações de

menor custo que promovam maior rentabilidade à produção (DJOUVINOV; MIHAILOV, 2005; ALAGAWANY; EL-HINDAWY; ATTIA, 2014). No entanto, dietas com níveis reduzidos de PB só são benéficas se o desempenho puder ser mantido (KARAALP, 2009).

A redução progressiva da PB da dieta pode induzir uma situação em que os aminoácidos se tornam limitantes para suportar um melhor desempenho (LIMA et al., 2013). O nível de um único aminoácido essencial que é deficiente pode resultar em uma dieta que não otimiza a eficiência econômica do sistema de produção (LIMA et al., 2016). Portanto, o equilíbrio entre os principais aminoácidos se torna necessário.

Atualmente, diversos estudos têm sido realizados com o objetivo de reduzir os efeitos adversos de dietas com altos níveis de PB (JORDÃO FILHO et al., 2012; ALAGAWANY et al., 2014; SANTOS et al., 2016). Os alimentos têm sido frequentemente combinados com a adição de pequenas quantidades de aminoácidos industriais para atender às necessidades das aves quanto aos aminoácidos mais limitantes. Dessa forma, as dietas podem ser formuladas com níveis reduzidos de PB, mas que ainda atendam as necessidades de aminoácidos e com o fornecimento de concentrações apropriadas de aminoácidos essenciais, evitando excessos (ALAGAWANY et al., 2014). Entretanto, a redução de PB à níveis superiores a 3%, mesmo que atenda a todos os requisitos de aminoácidos, resulta em baixo desempenho produtivo (JARIYAHATTHAKIJ et al., 2018). Sendo assim, é necessário primeiramente determinar as reais necessidades de PB dietética para que depois sejam feitos ajustes com aminoácidos industriais.

Uma estratégia que poderia ser utilizada para codornas japonesas é fornecer níveis adequados de PB com base nos períodos de crescimento como ocorre em frangos de corte e perus. À medida que as aves envelhecem o consumo de ração aumenta e o potencial de crescimento diminui. Portanto, há maior aporte de aminoácidos do que o necessário (KARAALP, 2009). As tabelas de exigências nutricionais do NRC (1994) e de Rostagno et al. (2011) apresentam recomendações de PB na dieta de codornas japonesas apenas para duas fases, uma para a cria e recria e outra para produção. No entanto, ao atualizarem suas tabelas nutricionais Rostagno et al. (2017) já recomendam dietas distintas para as fases de cria e recria, totalizando três níveis de PB ao longo da criação de codornas japonesas.

Embora as tabelas de exigências recomendem níveis adequados de PB na dieta de codornas, pesquisas na literatura apontam resultados bastante divergentes. Dessa maneira, estudos devem ser realizados, principalmente com codornas japonesas, para determinar o

melhor nível de PB que proporcione maior eficiência produtiva e reprodutiva das aves (ALAGAWANY et al., 2014).

2.2 Influência da proteína bruta na dieta sobre a atividade reprodutiva das fêmeas

O melhoramento genético têm promovido melhoras consideráveis no desempenho produtivo de codornas japonesas nos últimos anos (REDA et al., 2015). Atualmente, as codornas apresentam o crescimento mais rápido e maior eficiência de produção, tornando-se mais pesadas, mais produtivas e com ovos maiores (ROSTAGNO et al., 2017; HASANVAND et al., 2018). No entanto, a falta de padronização das linhagens em virtude desse constante trabalho de melhoramento, tem contribuído para as variações dos resultados de desempenho (ROSTAGNO et al., 2017).

Na literatura podem ser encontradas recomendações de PB na dieta que vão de 20% a 28% para as fases de cria e recria (MURAKAMI et al., 1993; LEESON; SUMMERS, 2009) e de 16% a 24% para a fase de produção (SIYADATI et al., 2011; ALAGAWANY et al., 2014). Portanto, as exigências ainda não estão bem estabelecidas, sendo necessários mais estudos para elucidar o melhor nível proteico para o máximo desempenho de codornas japonesas (GHAZAGHI et al., 2012).

Sabe-se que níveis adequados de proteína na dieta são importantes para promover maior desenvolvimento corporal e, consequentemente, maior desenvolvimento dos ovários nas fêmeas. O maior desenvolvimento do ovário pode representar maior peso do estroma e maior número de folículos. Uma vez que esses folículos apresentam capacidade esteridogênica, o desenvolvimento precoce dos mesmos pode adiantar a maturidade sexual das aves (RENEMA et al., 1999), trazendo benefícios econômicos para o produtor.

O óvulo das aves, diferente do que ocorre em mamíferos, acumula grande quantidade de lipídeos e proteínas em seu interior no momento em que o folículo é recrutado para a hierarquia folicular, pois este é utilizado como fonte nutritiva para o embrião caso ocorra a fertilização (SPEAKE; MURRAY; NOBLE, 1998; GONZALES, 2013; JOHNSON, 2014). A gema, como é comumente denominado, é composta por aproximadamente 34% de proteína e 66% lipídeos, com base na matéria seca (SCANES, 2014). No entanto, o ovário não apresenta capacidade de sintetizar os lipídeos e proteínas incorporados pelo oócito em desenvolvimento. Sendo assim, a maior parte desses nutrientes é sintetizada pelo fígado e transportada através

da corrente sanguínea até os ovários (SPEAKE; MURRAY; NOBLE, 1998; GONZALES, 2013).

O fígado é estimulado principalmente pelo estrógeno sintetizados pelas células da teca do folículo ovariano (OKULIAROVA; MEDDLE; ZEMAN, 2018). Uma vez que os pequenos folículos apresentam capacidade esteroidogênica, o aumento precoce das concentrações de 17 β-estradiol no plasma estimula o aumento das taxas de síntese de gema no fígado (RENEMA et al., 1999). Se o desenvolvimento dos pequenos folículos ocorre mais cedo devido ao maior aporte de aminoácidos para o desenvolvimento da ave, a maturidade sexual dessas aves pode ser antecipada. Soares et al. (2003) observaram que codornas alimentadas com 18% de PB durante a fase de cria e recria iniciaram a postura cerca de quatro dias depois do que as codornas alimentadas com 20, 22 e 24% de PB.

O aumento das concentrações de 17 β-estradiol no plasma em consequência do maior número de pequenos folículos no ovário, ao aumentar as taxas de síntese de gema no fígado, pode resultar também em maior tamanho de gema de ovo quando as mesmas já estão em produção (RENEMA et al., 1999). Diversos estudos têm mostrado que o aumento dos níveis de PB da dieta tem aumentado o peso e a porcentagem de gema (DJOUVINOV; MIHAILOV, 2005; REIS et al., 2011; ALAGAWANY et al., 2014; AGBOOLA et al., 2017). Lima et al. (2016) e Ozdemir e Inci (2012) também observaram que codornas mais pesadas apresentaram maior intensidade de postura e maior peso dos ovos, respectivamente. O aumento nos níveis de proteína, portanto, influencia o crescimento das codornas com consequências diretas no desenvolvimento do trato reprodutivo e subsequente produção de ovos.

Apesar de a manipulação dietética ter grande influência sobre a síntese de gema, a proporção dos componentes internos praticamente não é alterada. Isso se deve, principalmente, à forma de transporte de nutrientes do fígado para a gema, através da VLDLy (*very low density lipoprotein*) e vitelogenina, que são muito resistentes às variações (SPEAKE; MURRAY; NOBLE, 1998). Sendo assim, dietas com baixos níveis de PB podem reduzir o peso do ovo pela falta de nutrientes necessários para sua síntese, com pouca influência em sua composição.

A proteína da ração, quando deficiente, limita a síntese dos componentes do ovo e as aves podem compensar reduzindo o tamanho e o número de ovos ou aumentando o intervalo de postura (AGBOOLA et al., 2017). Diversos estudos têm mostrado que o aumento dos níveis de PB da dieta tem aumentado a produção (DJOUVINOV; MIHAILOV, 2005; REIS et al., 2011; ALAGAWANY et al., 2014; AGBOOLA et al., 2017), o peso e a massa de ovos

(AGBOOLA et al., 2017; RATRIYANTO; INDRESWARI; NUHRIAWANGSA, 2017). A deficiência desses nutrientes pode até chegar a comprometer toda a vida produtiva da ave, ocasionando na redução do pico e da persistência de postura, o que leva ao descarte e à reposição do plantel mais cedo (SILVA, 2003).

O albúmen possui como uma das suas principais funções o fornecimento de água e proteínas ao embrião. Esse componente representa cerca de 67% do peso do ovo, sendo que, em média, 85 e 91% de sua composição na matéria seca corresponde à PB em galinhas e codornas, respectivamente (NOVAK; YAKOUT; SCHEIDELER, 2006; GENCHEV, 2012). Mais de 40 proteínas diferentes estão presentes no albúmen, embora sete delas perfaçam mais do que 90% do total (RUTZ; ANCIUTI; PAN 2005). As principais são a ovoalbumina, que corresponde a 54% do total, ovotransferrina que corresponde a 13%, ovomucoide que corresponde a 11%, ovoglobulinas 8%, lisozima 3,5% e ovomucinas que perfazem menos de 3% do total de proteínas do albúmen (ITO; MIYAJI; MIYAJI, 2013).

Por ser rico em proteínas, o albúmen pode também ser influenciado pelos níveis de PB da dieta (GENCHEV, 2012). Novak, Yakout e Scheideler (2006) observaram que a percentagem de albúmen reduziu linearmente quando a ingestão de proteína diminuiu, sendo esse um dos prováveis responsáveis pela redução no peso do ovo. Segundo os autores, a diminuição na porcentagem de albúmen pode ter sido o resultado de uma diminuição na síntese do mesmo. Isso porque a rápida síntese de proteínas no magno faz com que o albúmen seja muito sensível às variações na concentração de aminoácidos no sangue e no fígado (REIS et al., 2011). Portanto, o fator de grande influência na síntese de albúmen são os níveis de PB presentes na dieta (ITO; MIYAJI; MIYAJI, 2013).

A casca do ovo, que é composta basicamente de carbonato de cálcio, tem como função principal a proteção do embrião contra agressões externas e a penetração de microorganismos, além de manter um ambiente interno muito bem controlado em termos de temperatura, pH, equilíbrio eletrolítico e oxigenação. A casca fornece ainda o cálcio e outros elementos necessários para o desenvolvimento do embrião, principalmente para a calcificação dos ossos (GONZALES, 2013).

A taxa de deposição desse componente parece ser menos influenciada pelos diferentes níveis dietéticos desse nutriente (NOVAK; YAKOUT; SCHEIDELER, 2006; AGBOOLA et al., 2017). No entanto, a espessura da casca pode ser reduzida com o aumento dos níveis de PB, porém, essa alteração se deve ao maior tamanho do ovo, que não é acompanhado por

maior deposição de casca. Portanto, as proporções dos componentes do ovo ficam modificadas (NOVAK; YAKOUT; SCHEIDELER, 2006; REIS et al., 2011).

Diante do exposto é possível observar que a produção de ovos é dispendiosa em termos de energia e proteína para as aves. Isso porque todos os nutrientes necessários para o crescimento e desenvolvimento do embrião devem estar presentes no ovo, além de estoques de energia para o processo de eclosão (YADGARY; YAIR; UNI, 2011; EVERAERT; WILLEMSEN; DECUYPERE, 2017).

Para a produção dos ovos, além da dieta, as aves podem também utilizar as reservas corporais como fonte de nutrientes. No entanto, a dieta é mais utilizada pelas codornas, uma vez que as reservas corporais podem não ser suficientes para atender a demanda (AGBOOLA et al., 2017). Cada um desses constituintes é de extrema importância para o correto desenvolvimento do embrião, havendo pouca possibilidade de sobrevivência caso haja a má formação de algum dos mesmos (GONZALES, 2013). Portanto, se o fornecimento de nutrientes pela dieta é deficiente, a produção de ovos pode ficar comprometida, assim como a composição interna do ovo, o que reduz o número de ovos incubáveis e a eclodibilidade dos mesmos (SCANES, 2014), reduzindo os índices produtivos do plantel.

Os prejuízos promovidos pelo fornecimento de dietas com níveis desbalanceados de PB para codornas em postura, no entanto, não se restringem apenas aos níveis abaixo da exigência. O excesso de consumo de proteína também é prejudicial à produção, assim como observado em outras aves comerciais. Altos níveis de PB na dieta podem levar à obesidade e maturidade sexual precoce. Como consequência pode haver aumento na incidência de ovos com gemas múltiplas, de prolapso de oviduto e redução da produção de ovos (SILVA, 2003).

Durante o período em que as fêmeas estão atingindo a maturidade sexual há naturalmente um acúmulo de gordura corporal, principalmente nas vísceras como fígado, ovário e oviduto para atender a demanda de produção dos ovos (SILVA et al., 2004). No entanto, o fornecimento de quantidade elevadas de PB na dieta podem levar ao acúmulo excessivo de gordura, reduzindo a capacidade produtiva da ave. Sendo assim, mais estudos são necessários para compreender o metabolismo proteico relacionado ao desenvolvimento do trato reprodutivo de fêmeas e sua influência na formação do ovo.

2.3 Influência da PB na dieta sobre a atividade reprodutiva dos machos

A maioria das tabelas de exigências nutricionais atuais é baseada em estudos de desempenho das aves comerciais predominantemente de fêmeas. Uma vez que machos e fêmeas apresentam importantes diferenças fisiológicas e metabólicas, é de se esperar que ocorram diferenças também nas necessidades nutricionais entre essas diferentes categorias de animais.

Na prática, os matrizeiros muitas vezes resistem em adotar um programa nutricional exclusivo para machos sob a alegação de dificuldades de manejo e do não convencimento da melhoria do desempenho reprodutivo (BORGES et al., 2006). Entretanto, o manejo apropriado dessa categoria sexual e o conhecimento das suas reais necessidades nutricionais, principalmente de PB, é de fundamental importância para a máxima produtividade do plantel (SOARES et al., 2003). Diversos estudos têm demonstrado que o correto desenvolvimento corporal dos machos à partir do fornecimento de dietas adequadas à essa categoria têm grande influência na melhoria da fertilidade do plantel, o que pode resultar na melhora dos índices produtivos e rentabilidade da produção (BORGES et al., 2006; SARABIA FRAGOSO et al., 2013; SILVEIRA et al., 2014).

A maior parte dos problemas reprodutivos de um plantel está relacionada a fatores ligados ao macho, sendo que o perfil de crescimento dos reprodutores é um dos principais determinantes da fertilidade de um lote (BONGALHARDO, 2013). Além disso, segundo esses mesmos autores, os machos, por estarem presentes em menor proporção em relação ao número de fêmeas dentro de um matrizeiro, um único macho com baixa fertilidade pode afetar o desempenho reprodutivo de maior número de fêmeas, reduzindo o número de ovos férteis.

A condição física corporal e as condições nutricionais apresentam grande influência no volume testicular e, consequentemente, nas concentrações plasmáticas de testosterona (AMOROSO et al., 2008; SARABIA FRAGOSO et al., 2013). O peso testicular, por sua vez, está diretamente relacionado com a capacidade de produção espermática (FRANÇA; RUSSELL, 1998). Isso porque o número de células de Sertoli no testículo adulto influencia a produção diária de espermatozóides. Essa relação ocorre devido a cada célula de Sertoli ter uma capacidade fixa para o número de células germinativas que pode suportar, embora essa capacidade varie entre as espécies (SHARPE et al., 2003). Portanto, a produção por grama de testículo é relativamente constante entre as espécies e como consequência, machos com maior

massa testicular apresentam maior capacidade de produção de espermatozoides. Sendo assim, a avaliação do peso testicular durante a idade reprodutiva é importante, pois é considerado um indicativo de fertilidade (LANNA et al., 2013).

As células de Sertoli proliferam apenas quando ainda se encontram imaturas, o que ocorre antes da puberdade, portanto, o número final de células de Sertoli é determinado antes da idade adulta. Os hormônios são importantes fatores que determinam o número de células de Sertoli por testículo, especialmente o FSH, mas outros hormônios ligados ao crescimento, como a testosterona podem contribuir (SHARPE et al., 2003). Além disso, a testosterona também é responsável pela diferenciação das células da linhagem espermatogênica e a produção espermática (LIAO et al., 2012). Dietas que promovam o desenvolvimento testicular precoce e aumento das concentrações de testosterona plasmática podem ter efeitos na maturidade sexual e na capacidade de produção espermática.

As características anatômicas da glândula da cloaca também é outro indicavo de fertilidade dos machos. A taxa de crescimento desse órgão, bem como a produção de espuma, é paralela à taxa de crescimento dos testículos. Dessa forma, a glândula pode ser considerada um bom indicador do tamanho e atividade testicular (RESHAG et al., 2011). De fato, ela é bem desenvolvida nos machos sexualmente ativos (KLEMM et al., 1973). Existe ainda forte correlação entre seu tamanho com frequência de liberação e peso de espuma, concentração de testosterona, atividade espermatogênica e fertilidade (OTTINGER; BRINKLEY, 1979; BISWAS et al., 2007; RESHAG et al., 2011).

O exsudato espumoso produzido pela glândula da cloaca é formado por glicoproteínas, lactato e diferentes enzimas comumente encontradas no sangue. Esses componentes presentes na espuma fornecem energia e aumentam a taxa metabólica dos espermatozoides, estimulam a motilidade e auxiliam na desagregação espermática, além de melhorar o transporte de espermatozoides no oviduto (SINGH et al., 2011). A motilidade, assim como o vigor, determina a capacidade dos espermatozoides para atingirem e fecundarem os óvulos no infundíbulo, sendo, portanto, um importante indicador de qualidade espermática (BORGES et al., 2006). Dietas que promovam o correto desenvolvimento da glândula da cloaca e a síntese do exudato espumoso podem melhorar a qualidade do sêmen dos machos.

A fertilidade dos machos está relacionada não só à qualidade do sêmen, mas também a fatores físicos, como capacidade de acasalamento que, juntos, influenciam o número de ovos férteis (FONTANA; WEAVER; KREY, 1990; TYLER; BEKKER, 2012). Sendo assim, o

ganho de peso durante as fases de crescimento deve ser controlado a fim de evitar redução na fertilidade do lote.

O acúmulo de gordura na carcaça proporcionado pelo excesso de nutrientes da dieta também pode influenciar na fertilidade dos machos devido à redução da qualidade espermática. Reprodutores que não produzem sêmen tendem a apresentar maior percentual de gordura abdominal em relação aos que estão produzindo altas quantidades (WILSON et al., 1987; BORGES et al., 2006). Borges et al. (2006) observaram que o aumento de deposição de gordura na carcaça reduziu o volume de sêmen, a concentração espermática, o vigor e a motilidade dos espermatozoides e, consequentemente, reduziu a fertilidade de galos. Portanto, em reprodutores pesados, o ganho de peso é controlado desde as fases iniciais do desenvolvimento, objetivando reduzir problemas reprodutivos associados à seleção genética para o crescimento, como a dificuldade de acasalamento e a redução da qualidade do sêmen, o que pode ser feito usando sistemas de alimentação separados para machos e fêmeas (FONTANA; WEAVER; KREY, 1990; RENEMA et al., 1999).

O fornecimento de dietas para machos baseada nas exigências de fêmeas pode levar ao consumo elevado de nutrientes. Sabe-se que o peso corporal das aves é um dos principais requisitos que afetam as exigências de mantença (NRC, 1994). Em codornas japonesas, os machos adultos apresentam menores pesos corporais do que as fêmeas. Nesse caso, a exigência de mantença das fêmeas é maior. Djouvinov e Mihailov (2005) observaram que, aos 21 dias de idade, não houve diferenças no peso corporal entre machos e fêmeas de codornas japonesas. Entretanto, aos 28 dias, as fêmeas tinham 8 a 9 g de peso corporal a mais do que os machos e essa diferença aumentou para 19 a 24 g aos 42 dias. Em galos, Rostagno et al. (2017) recomendam em torno de 11,7% de proteína bruta e para matrizes pesadas 13,4%, o que comprova a necessidade de distinção das necessidades nutricionais entre os sexos.

O acúmulo de gordura, no entanto, não é só resultante do excesso de nutrientes. A proteína quando deficiente na dieta limita o crescimento das aves e, com isso, o gasto energético para crescimento e mantença é menor. Nesse caso, o excesso de energia das rações promove maior acúmulo de gordura na carcaça (WILSON et al., 1987; BORGES et al., 2006). Borges et al. (2006) observaram que tanto dietas com baixos níveis de PB quando o excesso resulta na redução da qualidade do sêmen e fertilidade de galos. Em codornas, estudos nesse sentido são escassos, porém existem evidências de que problemas decorrentes do acúmulo excessivo de gordura são semelhantes aos verificados em outras espécies (SILVA, 2003).

Dessa forma, o ajuste de uma dieta adequada na fase de cria e recria para machos de codornas japonesas é fundamental para garantir a fertilidade do lote.

Quanto ao fornecimento de diferentes níveis de PB na fase reprodutiva de codornas japonesas, Scanes (2014) relata que é provável que existam altas demandas de síntese proteica na espermatogênese. Entretanto, informações sobre as necessidades desse nutriente para o desenvolvimento e manutenção dos órgãos sexuais masculinos em codornas são escassas. Oliveira et al. (2000), avaliando o aumento de 16 para 20% de PB na fase de produção, no entanto, não observaram efeito sobre o peso absoluto e relativo dos testículos, área e altura do epitélio germinativo, diâmetro máximo e mínimo e número de figuras de meiose dos túbulos seminíferos.

O conhecimento das reais necessidades nutricionais dessa categoria animal, principalmente de PB, é de fundamental importância para a máxima produtividade do plantel (SOARES et al., 2003). Sendo assim, há a necessidade de estudos acerca das necessidades nutricionais dos machos reprodutores de codornas japonesas criados em condições brasileiras.

3 CONSIDERAÇÕES FINAIS

Frente à importância dos níveis de PB no desenvolvimento corporal de machos e fêmeas de codornas japonesas e sua influência no desempenho reprodutivo subsequente, o fornecimento correto desse nutriente nas dietas de crescimento e produção são de extrema necessidade para a máxima produtividade do plantel. Portanto, mais estudos devem ser conduzidos para se alcançar formulações dietéticas precisas que assegurem máxima produção de ovos, fertilidade e eclodibilidade, aumentando a eficiência produtiva de codornas de um dia.

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MANUSCRIPT 1

Characteristics of male and female Japanese quails (*Coturnix coturnix japonica*) in the growth and production stages fed diets with different levels of crude protein

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ABSTRACT

The objective of this study was to evaluate the growth and reproductive performance of male and female Japanese quails in the growth (1 to 35 days of age) and production (36 to 96 days) stages that were fed diets with different levels of crude protein (CP). For this, 300 one-day-old males were randomly housed in 30 cages (10 birds/cage) and 360 females of the same age in 10 cages (36 birds/cage) until the birds were 35 days of age (growth phase). The treatments tested at this phase were five CP levels in the diet (18, 20, 22, 24 and 26%), which were reduced by four percentage units for the production phase. At that time, the birds were redistributed in 40 laying cages, each with 9 females and 3 males. During all experimental periods, every three days, 12 birds of each sex of each CP level were individually weighed up to 60 days of age for the estimation of the growth parameters using the Gompertz curve. For the production variables, a completely randomized design with five treatments (dietary CP levels) and 8 replicates (cages) was used. In the males, there was a linear increase ($P<0.05$) in the growth maturity rate and a linear reduction ($P<0.05$) in the maximum growth interval up to the level of 22% CP. Higher weight at growth maturity ($P<0.05$) was obtained with 18% CP. There was no effect ($P>0.05$) of CP levels on the semen quality and fertility rate. In females, the highest CP levels (24 and 26%) reduced ($P<0.05$) the maturity rate and increased ($P<0.05$) the age of maximum weight gain. The level of 24% CP increased ($P<0.05$) the weight at maturity, while the level of 26% reduced ($P<0.01$) the feed conversion and age at first egg and at 50 and 95% laying. This same level increased ($P<0.01$) the egg mass at both 47 and 57 days of age. The highest intensity of laying at 47 days of age was obtained ($P<0.01$) with either 24 or 26% CP. Egg weight was reduced ($P<0.01$) only when 18% CP was used. CP levels in the diet influence the body development of both male and female quails. However, reproductive traits are only affected in females. The levels of 26% CP in the growth phase and 22% in the production phase are recommended.

Keywords: Egg production, fertility, growth curve, quail production, reproduction, semen quality

1. Introduction

Animal reproduction is considered one of the most important factors in animal production. Animal reproduction is directly influenced by several factors, including nutrition. Not only quality but also quantity of dietary crude protein (CP) can have a great influence on the performance of the animals (Borges et al., 2006). Several studies have shown that low CP diets may limit body development, including that of reproductive organs (Hashiguchi et al., 1998; Renema et al., 1999; Lima et al., 2016). This limitation can influence not only the age at which birds reach sexual maturity but also the productive and reproductive performance of animals. On the other hand, higher CP diets can raise feed costs, increase the excreta nitrogen content (Alagawany et al., 2014a) and even influence the kidney health and consequently the longevity of birds (Reddy et al., 2002). Thus, adjusting the CP content in the diet is fundamental to guaranteeing profitability in a production system.

In quail production, dietary CP requirements are not yet well established when considering different categories of birds. Most of the current recommendations (NRC, 1994; Silva et al., 2012; Rostagno et al., 2017) are based on the performance of commercial females, with their requirements extrapolated to males. In addition, the lack of standardization of quail lineages and studies carried out in different regions of the world has contributed to the variations of the performance results (Vali et al., 2005; Omidiwura et al., 2016). In the literature, recommendations can be found ranging from 20 to 28% CP for the growth phase (Leeson and Summers, 2009; Alagawany et al., 2014b) and from 20 to 24% CP for the production phase (Siyadati et al., 2011; Alagawany et al., 2014a). Because the requirements

are not yet well established, studies are needed to verify the influence of dietary CP level on the reproductive performance of Japanese quails (Ghazaghi et al., 2012).

In general, in adulthood, male quails are smaller than females. According to the NRC (1994), body weight is one of the main factors that affects bird requirements for maintenance. Therefore, it is assumed that the maintenance requirements of males may also be lower than that of females (Grieser et al., 2018). Thus, the use of similar diets for both sexes could exceed nutrient needs for males. In addition, it is known that males play an important role in breeding because their fertility is directly related to the reproductive performance of this category of birds. Thus, the provision of adequate diets to males can have a major influence on the production of day-old quails.

Another importance of knowing the real requirements of dietary CP is the correct application of the ideal protein concept in the formulation of diets. The use of this concept represents an important advance in reducing the emission of pollutants through excreta without compromising poultry performance (Alagawany et al., 2014a). This practice is based on the reduction of CP in the diet with the inclusion of crystalline amino acids to achieve the best amino acid ratio. However, reducing the CP levels by more than three or four percentage units, even if the all amino acid requirements are attended, results in a lower productive performance (Aftab et al., 2006; Jariyahatthakij et al., 2018). Thus, the use of the ideal protein concept depends on the precise knowledge of the CP requirements for the suitable use of industrial amino acids.

Due to the importance of males in breeding and the need to apply the concept of ideal protein in the formulation of quail diets, it is necessary to determine the best levels of dietary CP for different sex categories. Thus, the objective of this study was to verify the influence of different levels of CP in the diet on the growth and reproductive performance of male and female Japanese quails.

2. Material and Methods

2.1. Location, animals and experimental design

The experiment was conducted at the Department of Animal Science of the Federal University of Lavras, in Lavras, Brazil, between March and June 2018. All experimental procedures were approved by the Animal Ethics Committee (protocol 057/2017).

A total of 300 one-day-old male Japanese quails (8.3 ± 0.8 g) and 360 females (8.7 ± 0.5 g) were purchased from a commercial hatchery. The birds were housed separately in masonry shed during the growth phase (1 to 35 days) in groups of 10 males or 36 females in cages (50 cm wide × 70 cm deep × 25.5 cm high) equipped with linear trough feeders, nipple drinkers and excreta collection trays. The temperature and humidity of the environment were monitored with minimum and maximum thermohygrometers positioned at the height of the birds in the central region of the shed. The temperature was maintained at 38 °C for the first three days with the use of a wood-burning heater and reduced 0.5 °C per day until the 28th day of the experiment (Albino and Barreto, 2003). The luminosity was 24 h of light (natural + artificial with 60 W incandescent lamps) during the first two days (24L0D), 23 h up to the 15th day (23L1D) and 14 h until the end of that phase (14L10D). During this period, the birds received experimental diets with different CP levels (18, 20, 22, 24 and 26%) with five male cages and two female cages for each diet.

For the production phase (36 to 96 days), the birds were weighed and transferred to a laying shed. In this phase, 9 females and 3 males were housed in each cage (32 cm wide × 38 cm deep × 16 cm high), totaling 40 experimental units. In this phase, the animals were distributed in a completely randomized design with five treatments (CP levels) and eight replicates (cages) per experimental unit. Starting from 36 days of the experiment, light was increased 30 min each day until 17 h light and 7 h dark (17L7D), was achieved, and this light

regime was used until the end of the experimental period (Faitarone et al., 2005; Barreto et al., 2007).

During the production phase, the birds were fed with the same experimental diets as during the previous phase, but with CP levels reduced by four percentage units (14, 16, 18, 20 and 22% CP), following recommendations (NRC, 1994; Rostagno et al., 2011). All experimental diets were isoenergetic and isonutrient, with the exception of CP (Tables 1 and 2) and were formulated with corn and soybean meal following recommendations (Rostagno et al., 2011). The CP levels were reduced or increased by two percentage units from the recommended level for both phases (22% for the growth phase and 18% for the production phase). Feed samples were taken for the evaluation of the real CP content. The diets were provided *ad libitum* during the 96 days of the experimental period.

2.2. Growth characteristics

For the determination of the growth curve, two males (12 in total) and six females (12 in total) from each cage of the growth phase were identified and weighed individually every three days from 1 to 60 days of age. The Gompertz model was used to estimate the weight at maturity of growth (A), growth rate (B) and age of maximum weight gain (M) of birds (Grieser et al., 2018). The growth curve was determined for each animal using the software Statistica version 13.3 (Statsoft Inc, Tulsa, USA) from the following model:

$$Live\ weight = A\ \times\ exp^{(-\exp^{(-B\ \times\ (M-day))})}$$

where A is the maturity weight, B is the growth rate and M is the maximum growth age (inflection point of the curve).

Each parameter of the Gompertz curve (A, B and M) was evaluated in a completely randomized design in a 5 × 2 factorial scheme (five CP levels and two sexes — males and

females), with six replications that considered the average between two quails obtained of the same experimental unit.

2.3. Performance of females

The daily egg production and weight of eggs were recorded from the first egg of each experimental unit up to 60 days of age. Laying intensity (number of eggs/number of birds/day × 100) and mass of eggs (g/bird/day) were calculated between 47 and 57 days, when the birds of the experiment reached 50 and 95% laying, respectively. Feed intake and feed conversion were also determined between 47 and 57 days of age. The feed conversion was calculated from the feed intake and egg mass produced.

2.4. Performance of males

From the 50^{th} day of the experiment, every two days, the males were submitted to the technique of semen collection through a dorso-abdominal massage. The sperm quality was evaluated in one male from each cage at 90 days of age.

Semen was evaluated with or without the presence of foam (Biswas et al., 2013). Before the semen collection period, the cloaca gland foam of each male was collected on days 80, 81 and 82 of the experiment, twice a day, always at 08:00 and 16:00, by light digital pressure on both sides of the gland. After collection, the foam was stored in falcon tubes and stored in a freezer at -20 °C. On the 82^{nd} day of the experiment, samples obtained on previous days were thawed at room temperature and diluted in saline solution (0.9%) in a ratio of 1:4 (foam:saline) and centrifuged at 3000 × g for 45 min (Sorvall ST 16 Centrifuge, Thermo Fisher Scientific, Massachusetts, USA). The supernatant was collected and stored in a freezer at -80 °C until the day of sperm quality analysis.

At 90 days of age, the males were submitted to three semen collections at intervals of three days each, using the technique of Burrows and Quinn (1937). Before the beginning of the collection period, the feathers of the pericloacal region were removed. During collection, the foam was also removed to avoid contact with the semen. Massages on the dorsum of the birds, starting near the base of the wings and ending near the cloaca, were made. Six movements per animal were performed. Then, slight digital pressure at the base of the phallus and the ampoules of the vas deferens were performed for the output of the semen. The seminal content was collected in graduated capillary tubes. The volume was measured, and instantly, the semen was diluted in 0.9% saline solution or 0.9% saline + 5% foam solution (Biswas et al., 2010) in the ratio of one part semen to 98 parts solution.

Immediately after dilution, the sperm motility and the intensity of the movements were measured. The evaluations were blindly made by three evaluators in three subsamples placed between slides and glass coverslips at 37 °C and observed at 200× magnification under an optical microscope (Olympus CX31, Olympus, Tokyo, Japan). Sperm motility was expressed as the percentage of moving cells, and the intensity of movement was rated on a scale of 0 to 5, with 0 being the lowest intensity and 5 being the maximum intensity.

For sperm viability, one drop of semen was mixed with one drop of eosin-nigrosin (Blom, 1950) on glass slides and evaluated at 400× magnification under an optical microscope (Olympus CX31, Olympus, Tokyo, Japan). The number of live cells (no color) and dead cells (pink) were used to calculate sperm viability (live cells/total number of cells × 100).

2.5. Fertility test

On the 60^{th} day of the experiment, the eggs from each cage were collected; eggs that were cracked, broken and dirty, as well as those eggs that were very large or very small were

171 excluded. Collected eggs were labeled and stored for 24 h at 20 °C. After this time, the eggs
172 were weighed and individual eggs were selected based on average weight. Eight eggs per cage
173 were selected. The eggs were disinfected with formaldehyde solution (37%) and potassium
174 permanganate (99%) at a ratio of 2:1 (Oznurlu et al., 2016) and incubated at 37.5 °C and 60%
175 humidity (Ben-Ezra and Burness, 2017) in an automatic incubator (Luna 480, Chocmaster,
176 Piraquara, Brazil). Through the 15th day of incubation, automatic scroll mats rotated the eggs
177 every 2 h (Bhagat et al., 2012). After 21 days of incubation, the number of eggs that hatched
178 and the number of eggs that were fertilized but that had not hatched were counted for the
179 calculation of fertility as a percentage.
180
181 **2.6. Statistical analysis**
182 The variables obtained by the Gompertz curve and the performance of males and
183 females were evaluated for normality (Anderson-Darling), homoscedasticity (Breusch-Pagan)
184 and independence of errors (Durbin-Watson). In cases of nonsignificance, analysis of variance
185 (ANOVA) was performed, and CP levels were submitted to regression analysis. In cases of
186 lack of fit in the linear regression analysis (R^2<0.70), broken line analysis was performed to
187 determine the best CP level for each sex (Wen et al., 2016). When the ANOVA assumptions
188 were not met and the Box-Cox and Johnson data transformations were not effective in
189 normalizing the data, the data were subjected to nonparametric analysis and the means
190 compared by the Kruskal-Wallis test. Statistical analyses were performed using the software
191 Statistica version 13.3 and Action version 3.5.
192
193
194

3. Results

3.1. Body development

The growth characteristics of the quails were different between males and females (Figure 1). Females had a lower (P<0.01) growth maturity rate (late maturity), greater weight at growth maturity, and greater age at which maximum growth occurred (Table 3).

The dietary CP levels influenced (P<0.05) the growth curve of males and females in different ways. In both groups, there was a CP × sex interaction (P<0.01) in the growth maturity rate and age of maximum weight gain. In males, the increase in CP level up to 22.2% in the growth phase linearly reduced (P<0.01) the age of the maximum weight gain and antecipated (P<0.01) the body growth maturity (Figure 2), resulting in lighter animals at the end of growth when 20% CP or more were used. In females, the increase of CP levels linearly increased (P<0.01) the age of the maximum growth and delayed the age at growth maturity when 24 and 26% CP were used. Females that received 24% CP presented higher weight at growth maturity.

Regarding live weight, there was no interaction (P>0.05) between CP × sex (Table 4). Females showed higher live weight (P<0.05) when compared to males at 35 days of age. The dietary CP levels linearly increased (P<0.01) the weight of the birds until 49 days of age. The *plateau* model indicated that the optimal CP level for greater live weight was 23.7% from 1 to 35 days of age and 19.4% from 36 to 60 days (Figure 3). At 60 days of age, a linear effect of dietary CP levels was observed only in females (P<0.01).

3.2. Reproductive performance

The reproductive performance of males at 90 days of age was not influenced (P>0.05) by CP levels in the diet (Table 5). In females, the CP content linearly reduced (P<0.05) the age of first egg laying and the ages of 50 and 95% laying. Broken line analysis indicated that

this linear effect occurred up to 24% CP in the growth phase and 20% in the production phase (Figure 4). The intensity of laying at 47 days of age was higher ($P<0.01$) for the highest dietary CP levels (24 and 26% in the growth phase and 20 and 22% in the production phase). At 57 days, the intensity of laying was similar between the different CP levels, except when 18% CP in the growth phase and 14% in the production phase were used. In this case, there was a reduction ($P<0.01$) in the intensity of laying of the birds at that age. A similar result was observed in egg weight. Both at 47 and 57 days of age, the lower CP level reduced ($P<0.01$) the egg weight. On the other hand, higher CP levels (26% in the growth phase and 22% in the production phase) increased ($P<0.01$) the egg mass in relation to the other CP levels. There was no effect ($P>0.05$) on feed intake. The feed conversion linearly reduced ($P<0.01$) with increasing CP levels in the diet.

4. Discussion

Although body development was influenced by dietary CP levels in both females and males, only the reproductive performance of females was affected. For this sex category, the 26% CP level in the growth phase and 22% in the production phase are recommended to maximize production.

Dietary CP is an important source of amino acids for animals. The quality of CP in the diet is related not only to the relationship between the main amino acids but also to the digestibility of the forms and the amounts in which they are offered (Siyadati et al., 2011). Both the excess and the lack of amino acids are related to the use of these nutrients by the tissues. Imbalance influences the metabolic and physiological functions of animals and, consequently, their reproductive efficiency.

In quail production, CP requirements are not yet well established, especially for males. In breeding, reproductive efficiency is represented by the production of fertile eggs. This

characteristic is more influenced by males than by females, since the performance of a single male can affect the reproductive efficiency of three females (Lotfi et al., 2018). In this case, adjustment of dietary CP levels for both sex categories could increase the one-day-old chick production. However, the results of the present study showed that dietary CP levels influenced male body development but not reproductive efficiency. This suggests that the lower CP level used (18% in the growth phase) should be used since low CP diets are associated with lower nitrogen elimination and lower feed costs (Alagawany et al., 2014b). However, due to management difficulties in breeding production, adopting a nutritional program exclusively for males is uncommon (Borges et al., 2006). Therefore, based on the results of the present study, diets formulated based on the CP requirements of the females can be used to feed the males without affecting to the fertility of the quail production establishment.

Regarding body development, dietary CP levels differently influenced considering males and females separately. In males, the lowest CP levels (18% in the growth phase and 14% in the production phase) increased the time that the birds presented maximum growth, delayed the growth maturity rate and, consequently, resulted in heavier birds at the end of the body development. In females, the 24% CP diets resulted in higher body development. According to the NRC (1994), body weight is directly related to maintenance requirements. In this case, the results obtained in the present study may be related to the body difference between male and female quails. This difference was significantly detectable from 35 days of age (Table 4). Considering the growth characteristics obtained by the Gompertz curve (Table 3), it is possible to observe that lower CP diets resulted in a greater range of maximum growth in males (22 days), with a consequent delay in male growth maturity (lower rate of maturity). This result may be related to the compensatory gain presented by males during the last week of growth (27 and 35 days of age) to the third week of the production phase (56 days of age).

At this time, the live weight of the animals fed diets with different CP levels was similar. In females, compensatory gain also occurred up to 56 days of age, but it was not enough to maintain body development up to 60 days of age, as occurred with males. It is important to note that, at this age, the birds were already at their peak of production. In other words, lower CP diets may have limited the body development of the quails since part of the amino acids may have been used for egg production (Allen and Young, 1980; Silva et al., 2004). This explains the linear increasing effect of dietary CP levels on the live weight of females at 60 days of age.

In the present study, the live weight of the females began to differ from the live weight of males as early as 35 days of age, when the animals normally move to the production stage. Djouvinov and Mihailov (2005) observed that at 28 days of age, females were 8 to 9 g heavier than males, and this difference increased to 19 to 24 g at 42 days of age. As females have a greater weight in adult life, it is normal that they achieve a later growth maturity associated with lower maturity rates in relation to males (Djouvinov and Mihailov, 2005; Grieser et al., 2018). In addition, the targeting of amino acids for egg production limits the body development of animals, also contributing to the delay in the growth maturity of the females. However, in females that were fed with the lowest dietary CP level, the compensatory gain occurred through approximately 56 days (the age at which the live weight did not differ significantly between the CP levels); these birds were not able to achieve equal live weights at 60 days of age. In this case, the lack of amino acids may have limited the body development of the birds at that stage. This justifies the lower live weight of females fed diets with lower CP levels up to 60 days of age (Table 3).

As opposed to males, in females, CP levels influenced not only body development but also productive efficiency. The levels of 24% CP in the growth phase and 20% in the production phase were associated with higher body development and were also the optimal

levels to reduce the age at first egg and age at 50% and 95% of laying. These results were related to the onset of sexual activity which may be associated with greater anatomical development of the gonads. Renema et al. (1999) observed that heavier birds show early sexual maturity. These authors associated these results with the higher relative weights of ovary and oviducts and a greater number and weight of ovarian follicles. Lilburn et al. (1992) also observed an increase in the body weight of Japanese quails fed higher CP levels (24 vs. 30%) associated with reduced sexual maturity of birds.

In addition to the size of the sex organs, the availability of nutrients is also related to the reproductive efficiency of the birds. In the present study, higher dietary CP levels (26% CP in the growth phase and 22% in the production phase) resulted in higher intensity of laying at 47 days of age and higher egg masses at both 47 and 57 days of age. These results suggest that higher dietary CP may be necessary at the beginning of productive activity. During this phase, females are still growing. From that age, the nutritional requirements of birds may be lower. However, more studies using staple feeding programs for quails in the production phase should be performed.

The lower CP levels used (18% in the growth phase and 14% in the production phase) resulted in lower weight and lower egg mass. It is known that dietary CP, when deficient, may limit the synthesis of egg components. In this case, there may be a reduction in the size or number of eggs laid, or a restrictive diet may increase the range of laying (Agboola et al., 2016). In fact, several studies have shown the influence of dietary CP on the physical characteristics of quail eggs (Agboola et al., 2016; Ratriyanto et al., 2017). In the present study, higher dietary CP improved the feed conversion, proving that a higher amino acid intake increases the efficiency of utilization of the nutrients in egg composition.

In general, the optimum CP levels in the diet that conferred the highest live weight of the birds in the different stages of growth for the maximum production of females were 23.7%

up to 35 days of age and 19.4% up to 49 days of age. These results are close to those established by the NRC (1994) and Rostagno et al. (2017). However, higher CP levels may be required to increase egg mass without compromising productive efficiency. In addition, these levels can also be maintained for males without compromising the reproductive efficiency of these animals.

5. Conclusion

The CP content in the diet influences, in a differentiated way, the growth of male and female Japanese quails. However, reproductive characteristics are influenced only in females. Therefore, males can be fed diets based on the CP requirements of the females without affecting to the fertility of the male reproductive system. For maximum productivity, the levels of 26% CP in the growth phase and 22% in the production phase are recommended.

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Table 1

Centesimal composition and calculated nutritional levels of experimental diets for Japanese quails in growth stages (1 to 35 days).

Ingredients	Crude protein (%)				
	18	20	22	24	26
Corn	56.30	52.40	48.55	44.70	40.80
Soybean meal	28.80	33.89	38.95	44.03	49.10
Wheat bran	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.240	1.192	1.145	1.098	1.050
Limestone	1.35	1.35	1.35	1.35	1.35
Vegetal oil	3.50	3.58	3.65	3.73	3.80
Salt	0.399	0.399	0.398	0.397	0.397
Vitamin supplement[1]	0.100	0.100	0.100	0.100	0.100
Mineral supplement[2]	0.100	0.100	0.100	0.100	0.100
DL-methionine (99%)	0.005	0.038	0.070	0.103	0.136
L-lysine (78%)	0.052	0.039	0.026	0.013	0.000
L-threonine (99%)	0.002	0.015	0.031	0.046	0.060
L-tryptophan (98%)	0.004	0.003	0.002	0.001	0.000
Choline chloride (60%)	0.050	0.050	0.050	0.050	0.050
Kaolin	6.00	4.75	3.50	2.25	1.00
TOTAL	100.00	100.00	100.00	100.00	100.00
Nutritional composition calculated					
Metabolizable energy (kcal/kg)	2,900	2,900	2,900	2,900	2,900
Crude protein (%)	18.00	20.00	22.00	24.00	26.00
Crude protein analyzed (%)	18.32	20.09	21.92	24.14	26.39
Digestible lysine (%)	0.881	0.998	1.115	1.232	1.349
Digestible methionine + cystine (%)	0.500	0.566	0.630	0.696	0.761
Digestible threonine (%)	0.613	0.692	0.773	0.854	0.934
Digestible tryptophan (%)	0.202	0.229	0.256	0.282	0.309
Calcium (%)	0.900	0.900	0.900	0.900	0.900
Available phosphorus (%)	0.333	0.333	0.333	0.333	0.333
Sodium (%)	0.176	0.176	0.176	0.176	0.176
Amino acid/lysine ratio					
Lysine (%)	100	100	100	100	100
Methionine + cystine (%)	56	56	56	56	56
Threonine (%)	69	69	69	69	69
Tryptophan (%)	23	23	23	23	23

[1] Content per kg of feed (minimum for all elements): 10 mg of copper, 50 mg of iron, 1.2 mg of iodine, 80 mg of manganese, 0.28 mg of selenium and 60 mg of zinc.

[2] Content per kg of feed: 0.8 mg of folic acid, 35 mg of pantothenic acid, 1.0 mg of biotin, 40 mg of niacin, 11,500 IU of vitamin A, 3.0 mg of vitamin B1, 22 IU of vitamin E, 0.6 mg of vitamin B12, 4.4 mg of vitamin B2, 10.0 mg of vitamin B6, 2,100 UI of vitamin D3, 1.5 mg of vitamin K3, and 125 mg de antioxidant.

458 **Table 2**

459 Centesimal composition and calculated nutritional levels of feed for Japanese quails at the

460 production stage (36 to 96 days)

Ingredients	Crude protein (%)				
	14	16	18	20	22
Corn	61.10	57.05	53.00	48.95	44.90
Soybean meal	19.35	24.39	29.43	34.47	39.50
Wheat bran	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.153	1.107	1.06	1.014	0.967
Limestone	6.784	6.785	6.785	6.788	6.790
Vegetal oil	2.600	2.675	2.75	2.825	2.900
Salt	0.323	0.323	0.322	0.321	0.320
Vitamin supplement [1]	0.100	0.100	0.100	0.100	0.100
Mineral supplement [2]	0.100	0.100	0.100	0.100	0.100
DL-methionine (99%)	0.427	0.516	0.604	0.692	0.780
L-lysine (78%)	0.373	0.366	0.358	0.349	0.340
L-threonine (99%)	0.037	0.038	0.038	0.038	0.038
L-tryptophan (98%)	0.023	0.020	0.016	0.013	0.009
Choline chloride (60%)	0.037	0.037	0.037	0.037	0.037
Kaolin	4.50	3.45	2.30	1.20	1.00
TOTAL	100.00	100.00	100.00	100.00	100.00
Nutritional composition calculated					
Metabolizable energy (kcal/kg)	2,800	2,800	2,800	2,800	2,800
Crude protein (%)	14.71	16.71	18.71	20.71	22.71
Crude protein analyzed (%)	14.07	16.20	18.04	20.01	21.99
Digestible lysine (%)	0.809	0.927	1.058	1.162	1.279
Digestible methionine + cystine (%)	0.664	0.761	0.868	0.953	1.049
Digestible threonine (%)	0.485	0.556	0.638	0.697	0.767
Digestible tryptophan (%)	0.170	0.195	0.220	0.244	0.269
Calcium (%)	0.909	0.909	2.909	0.909	0.909
Available phosphorus (%)	0.303	0.303	0.303	0.303	0.303
Sodium (%)	0.176	0.176	0.145	0.176	0.176
Amino acid/lysine ratio					
Lysine (%)					
Methionine + cystine (%)	100	100	100	100	100
Threonine (%)	82	82	82	82	82
Tryptophan (%)	60	60	60	60	60
Metabolizable energy (kcal/kg)	21	21	21	21	21

461 [1] Content per kg of feed (minimum for all elements): 10 mg of copper, 50 mg of iron, 1.2 mg of iodine, 80 mg of

462 manganese, 0.28 mg of selenium and 60 mg of zinc.

463 [2] Content per kg of feed: 7,300 IU of vitamin A, 2,120 IU of vitamin D3, 8,500 IU of vitamin E, 2 mg of vitamin

464 K3, 1.1 mg of vitamin B1, 3.5 mg of vitamin B2, 2.1 mg of vitamin B6, 5.05 μg of vitamin B12, 25.5 mg of

465 niacin, 10 mg of pantothenic acid, 100 μg of biotin, and 18 mg of antioxidant.

Table 3

Growth characteristics of Japanese quails from 1 to 60 days of age estimated by the Gompertz curve. Quails were fed diets with different levels of crude protein in the growing (1 to 35 days: 18, 20, 22, 24 or 26% CP) and production (36 to 96 days: 14, 16, 18, 20 or 22% CP) phases.

Crude protein (%)		Weight at growth maturity (g)		Growth maturity rate		Age of maximum gain (days)	
		Male	Female	Male	Female	Male	Female
	18/14	159.3a	194.9a	0.055*	0.058a	22.8*	18.0**
	20/16	139.3b	201.3a	0.067	0.055a	17.3	18.7
	22/18	136.1b	191.7a	0.088	0.055a	12.8	19.5
	24/20	146.3b	215.8b	0.084	0.046b	13.1	23.6
	26/22	143.5b	195.9a	0.086	0.045b	12.9	26.6
P =	Sex	<0.01		<0.01		<0.01	
	Protein	0.19		0.02		0.09	
	Sex*Protein	0.08		<0.01		<0.01	
	Protein (within sex)	0.02	0.02	<0.01	<0.01	<0.01	<0.01
	SEM[1]	9.50		0.004		1.60	

[1] Standard error of the mean

[a,b] Means followed by different letters differ by the Scott-Knott test (P<0.05)

* Linear response plateau (P<0.05)

** Linear effect (P<0.01)

Table 4

Live weight of male and female Japanese quails fed diets with different levels of crude protein in the growing (1 to 35 days of age: 18, 20, 22, 24 or 26% CP) and production (36 to 60 days: 14, 16, 18, 20 or 22% CP) phases.

Age (days)	Crude protein (%) - male					Crude protein (%) - female					SEM[1]	P value		
	18/14	20/16	22/18	24/20	26/22	18/14	20/16	22/18	24/20	26/22		Sex	Protein	Sex*Prot
7	16.3*	19.5	22.9	22.7	24.6	17.8*	22.4	22.7	25.2	25.4	0.99	0.02	<0.01	0.53
16	40.5**	52.2	57.1	63.7	61.6	42.5**	51.4	54.7	63.0	58.9	2.49	0.56	<0.01	0.88
22	58.8**	72.6	79.7	86.1	87.6	62.7**	73.0	79.5	88.0	89.1	2.85	0.41	<0.01	0.96
28	77.2**	90.4	100.6	106.1	105.2	80.1**	90.9	98.7	105.7	109.0	3.21	0.63	<0.01	0.90
37	98.3**	98.9	106.2	119.4	112.2	99.3**	111.1	111.8	125.5	121.5	4.71	0.03	<0.01	0.80
43	124.6*	123.1	118.7	137.2	133.8	127.6*	144.9	144.9	158.9	155.4	4.78	<0.01	<0.01	0.14
49	129.3**	137.2	124.8	140.3	137.7	144.3**	164.5	161.3	169.2	168.3	4.46	<0.01	<0.01	0.20
58	139.3	134.4	129.1	140.4	138.6	161.2	170.5	164.2	174.3	175.1	4.29	<0.01	0.08	0.41
60	139.7	133.0	129.6	140.7	138.0	162.4*	174.8	169.9	180.6	177.5	3.88	<0.01	0.04	0.09

[1] Standard error of the mean

* Linear effect (P<0.05).

** Linear response plateau (P<0.05)

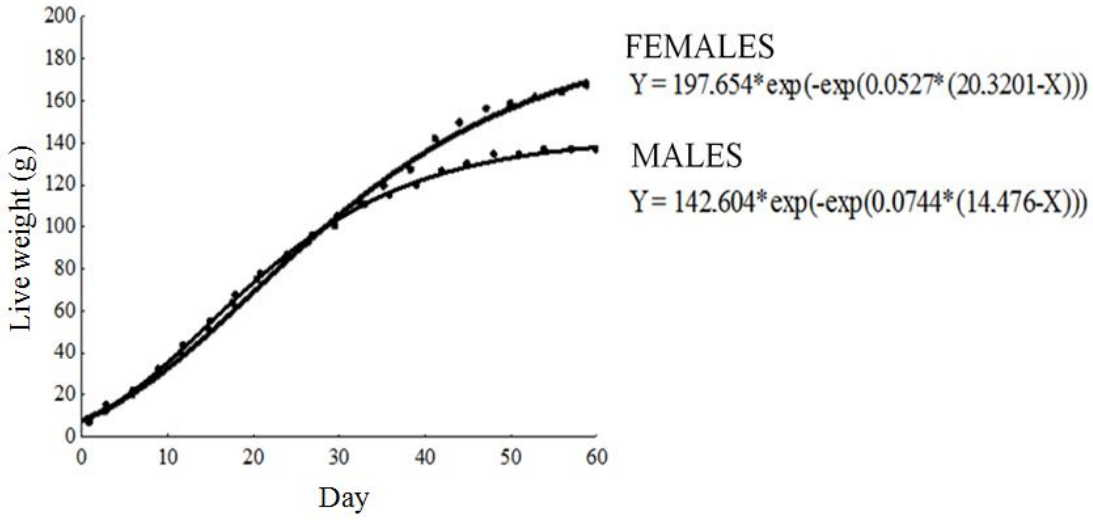
Table 5

Performance and reproductive characteristics of Japanese quails fed diets with different levels of crude protein in the growing (from 1 to 35 days of age: 18, 20, 22, 24 or 26% CP) and production (from 36 to 96 days of age: 14, 16, 18, 20 or 22% CP).

Variable	Crude protein (%) - growing/production phase					SEM	P value
	18/14	20/16	22/18	24/20	26/22		
			- Female-				
Age (days)							
1st egg	46.5*	44.1	42.5	41.4	41.1	0.81	<0.01
50% laying	51.4*	49.3	48.4	46.1	46.0	0.69	<0.01
95% laying	59.6*	56.4	53.3	48.4	48.6	0.99	<0.01
Intensity of laying (%)							
47 days of age	17.2a	38.4b	50.5b	73.4c	82.8c	-	<0.01
57 days of age	85.1a	94.3ab	98.5ab	96.4ab	100.0b	-	0.02
Egg weight (g)							
47 days of age	8.30A	9.28B	9.45B	9.84B	9.92B	0.21	<0.01
57 days of age	9.61A	10.66B	10.46B	10.67B	10.98B	0.13	<0.01
Egg mass (g/bird/day)							
47 days of age	1.19**	3.52	4.74	7.03	8.11	0.69	<0.01
57 days of age	8.01A	10.04B	10.10B	9.70B	10.98C	0.32	<0.01
Feed intake (g) (49-59 days)	210	221	214	219	219	3.73	0.14
Feed conversion (49-59 days)	4.97**	3.72	3.32	3.17	3.05	0.19	<0.01
			- Male -				
Volume (mL)	3.42	3.11	2.79	4.26	4.10	0.38	0.11
Semen without foam							
Motility (%)	72.7	77.1	68.8	74.3	74.0	2.61	0.57
Intensity of movements	3.08	3.39	3.29	3.37	3.27	-	0.68
Viability (%)	95.7	96.0	95.2	96.2	96.6	0.35[2]	0.39
Semen with foam							
Motility (%)	73.7	78.5	70.4	74.0	75.9	2.55	0.54
Intensity of movements	3.48	3.81	3.58	3.59	3.58	-	0.84
Viability (%)	94.8	95.9	96.9	96.9	96.4	0.34[2]	0.88
Fertility (%)	93.5	92.6	91.7	91.7	92.6	-	0.98

[a, b] Means followed by different letters differ by the Kruskal Wallis test (P<0.05)
[A, B] Means followed by different letters differ by the Scott-Knott test (P<0.05)
[2] Data transformed by the transformation of Box Cox
[3] Data transformed by Johnson's transformation
* Linear response plateau (P<0.05)
** Linear effect (P<0.05)
SEM: Standard error of the mean

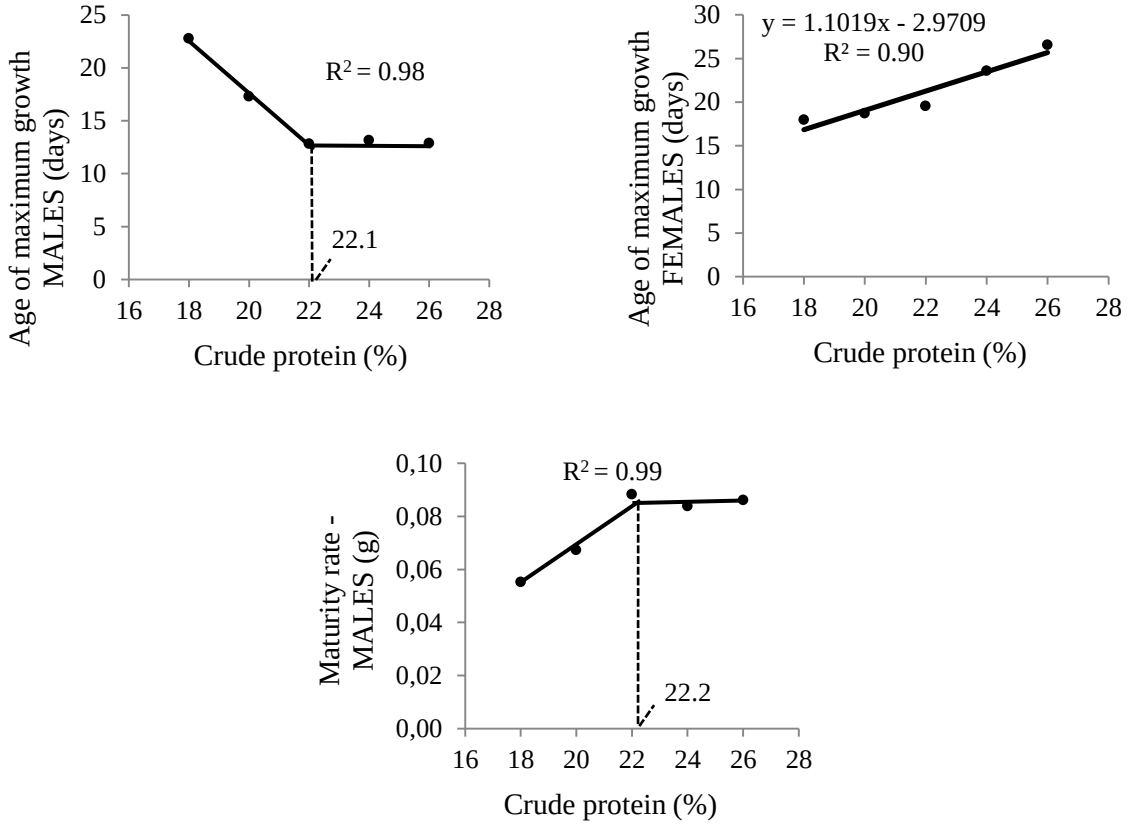
493



494

495 **Fig 1**

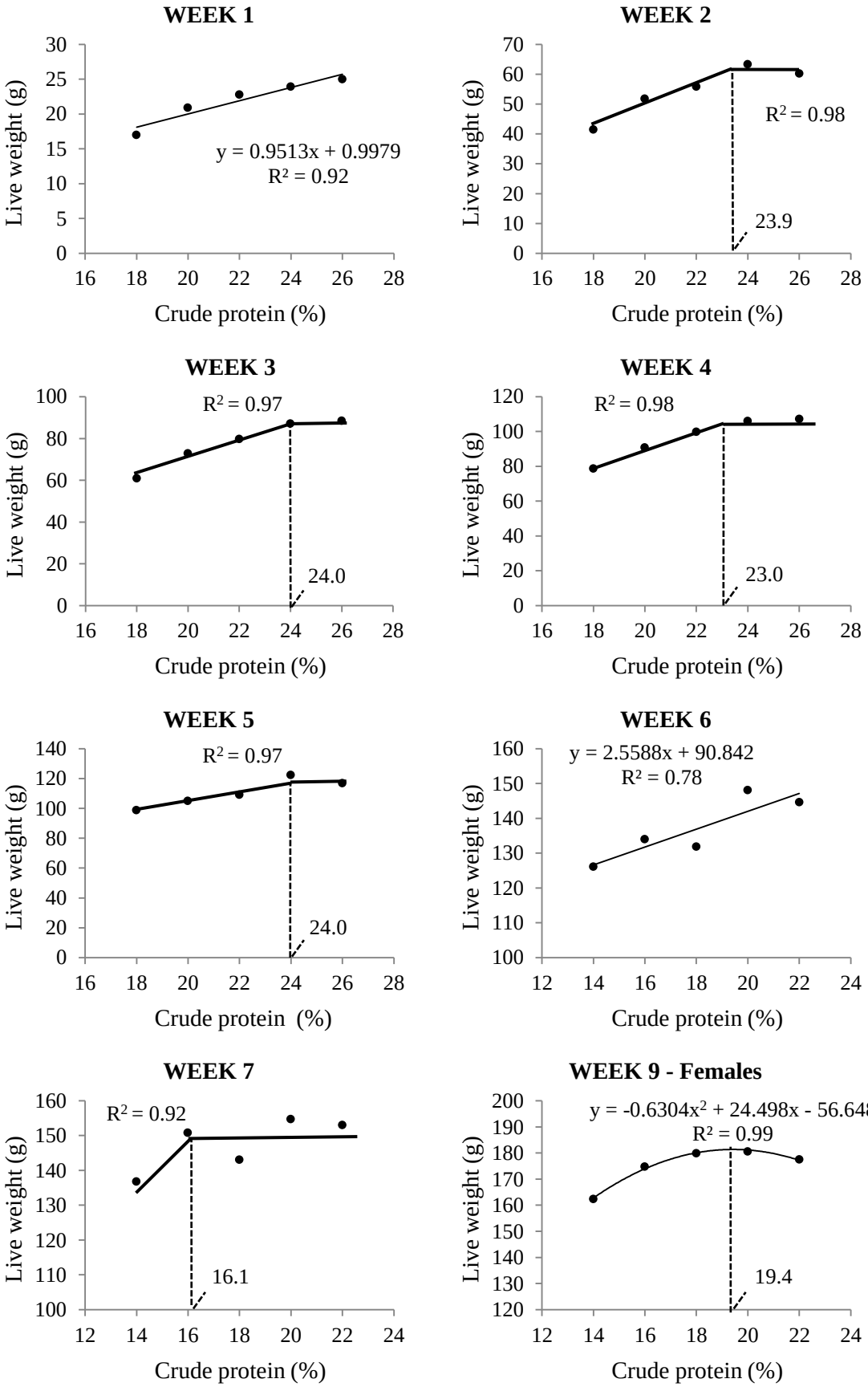
496 Growth curve of male and female Japanese quails fed diets with different levels of crude
497 protein in the growing (18, 20, 22, 24 or 26% CP) and production (14, 16, 18, 20 or 22% CP)
498 phases.



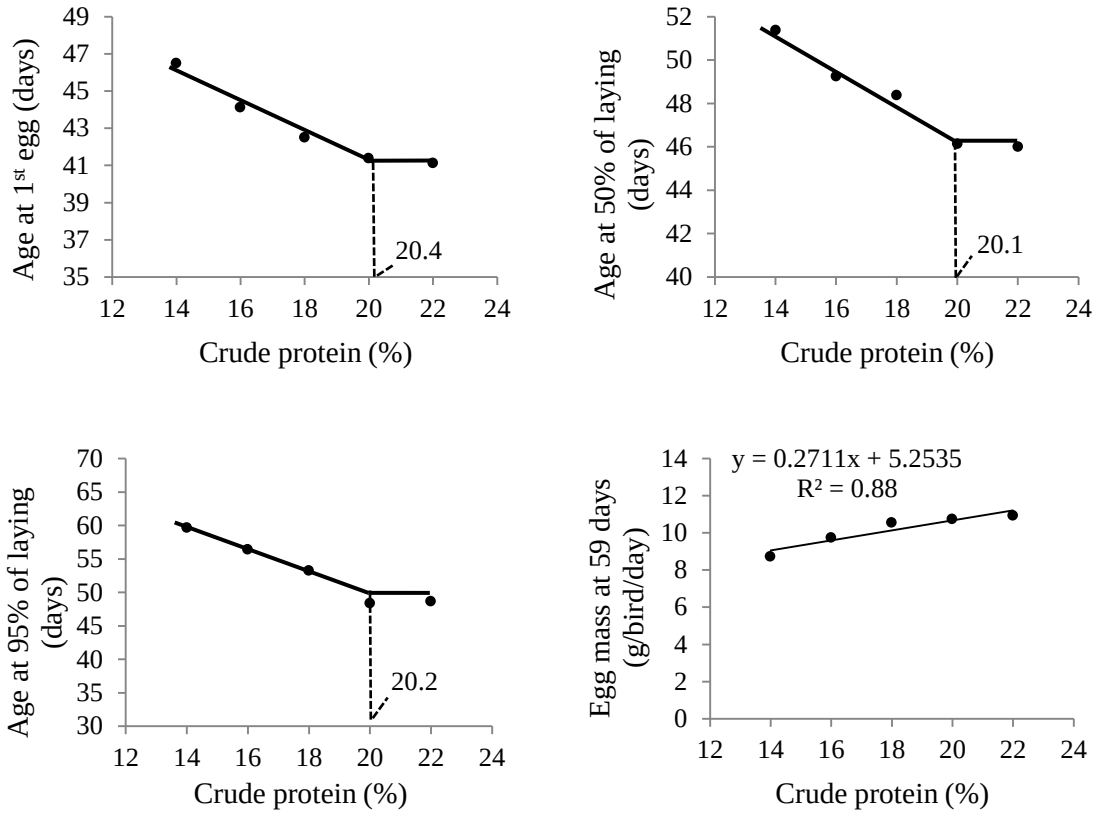
499 **Fig 2**

500 Growth patterns estimated by the Gompertz model of male and female Japanese quails fed

501 diets with different levels of crude protein.



502 **Fig 3** Live weight of male and female Japanese quails fed diets with different levels of crude
503 protein. Week 9: Not significant for males (P>0.05).



504 **Fig 4**

505 Performance of female Japanese quails fed diets with different levels of crude protein in the
506 growing (18, 20, 22, 24 or 26% CP) and production (14, 16, 18, 20 or 22% CP) phases. The X
507 axis is shows only the crude protein levels used in the production phase.

508

MANUSCRIPT 2

DIETARY CRUDE PROTEIN AND REPRODUCTION IN MALE JAPANESE QUAILS

Reproductive efficiency of male Japanese quails (*Coturnix coturnix japonica*) fed diets with different crude protein levels during the growth phase

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ABSTRACT The present study evaluated the influence of diets with different crude protein (CP) levels during the growth phase (1 to 35 days) on body development and reproductive characteristics in male Japanese quails. Three hundred male one-day-old quails were distributed between five treatments with different CP levels (18%, 20%, 22%, 24% and 26%) in a completely randomized design, with six replicates of 10 birds each. During the production phase (36 to 96 days), the birds were transferred to 30 laying cages, each with three males and nine females, and all birds received the same diet, formulated to meet production-phase requirements. The growth rate increased linearly ($P < 0.01$), and the age of maximum growth decreased ($P < 0.05$) with increasing dietary CP. No effect of CP level was observed on body weight at maturity ($P > 0.05$). With 23% CP, live weight increased only until 48 days of age ($P < 0.01$) and from then on presented no significant differences ($P > 0.05$). During the growth phase (1 to 35 days), weight gain was higher with diets containing 22% CP or higher. No effect on feed conversion was observed in this phase. Increased dietary CP increased nitrogen intake and excretion ($P < 0.01$) but did not affect nitrogen retention ($P > 0.05$). At this age, testis size, seminiferous tubular area, spermatogonia cell number and germinal epithelial height increased linearly ($P < 0.05$) with increasing dietary CP and the Leydig cell number decreased ($P < 0.01$). At 60 days of age, the Sertoli cell numbers increased linearly ($P < 0.01$) with increasing dietary CP. At 90 days of age, dietary CP did not affect cloacal gland size, foam weight and protein concentration, semen volume and quality or flock fertility ($P > 0.05$). In conclusion, dietary CP concentration affected body and testicular development in male Japanese quails without affecting their reproductive efficiency. A 23% CP level is recommended for better body development.

Key words: quail breeding, growth curve, testicular development, fertility, semen quality

INTRODUCTION

Quails are bred worldwide for both meat and egg production because of their ease of handling, small size compared with chickens and broilers, precociousness, small space requirements, low investment and high egg production capacity and rates (Minvielle, 2004). In addition, quail breeding has been used as a good experimental model for bird research because of the birds' rapid growth, greater egg production and shorter intervals between generations (Shit et al., 2010).

Nutrition is a main factor affecting quail productive efficiency. Dietary crude protein (CP) is an important source of amino acids, which are used for maintenance, growth, and production demands (Wei et al., 2011). During the growth phase, amino acid presence is directly related to the birds' body development (Renema et al., 1999; Lima et al., 2016), and both their excess and deficiencies may impair organ function as adults (Jordão Filho et al., 2012). Studies have shown that body development is highly correlated with reproductive organ development (Fontana et al., 1990; Lilburn et al., 1992). Delays in reproductive organ development caused by inadequate dietary CP levels may therefore be directly related to the birds' sexual maturity and reproductive performance (Renema et al., 1999; Lima et al., 2016).

Regarding quails' nutritional requirements, most current recommendations (NRC, 1994; Rostagno et al., 2011) are based on the performances of commercial unsexed or only female birds. However, since males and females present different growth characteristics, their nutritional requirements should also differ (Djouvinov and Mihailov, 2005). In one-day-old chick production systems, most reproductive problems are due to factors related to males because males are present in smaller numbers than are females (Bongalhardo, 2013). Thus, different feeding programs should be adopted for each sex to increase flock reproductive indices (Grieser et al., 2018). Studies with broiler breeders have shown that separate feeding arrangements for each sex resulted in improved reproductive indices (Fontana et al., 1990;

Silveira et al., 2014). Because male quails' requirements are unestablished, studies should evaluate the nutritional requirements of male Japanese quail breeders in the growth phase while they are housed separately from females. In addition, information on the protein requirements for sex organ development and maintenance in quails is scarce. Thus, the present study evaluated the influence of different dietary CP levels on male Japanese quail growth and flock reproductive parameters.

MATERIAL AND METHODS

This experiment was performed between March and June 2018 at the Department of Animal Science of the Federal University of Lavras (UFLA), Lavras, state of Minas Gerais, Brazil. The Animal Research Ethics Committee approved all experimental procedures under protocol no. 057/2017.

Three hundred male one-day-old Japanese quails (*Coturnix coturnix japonica*) were housed in a masonry shed in 30 cages (50 cm wide × 70 cm deep × 25.5 cm high), during the growth phase (1 to 35 days). For the production phase (36 to 96 days), the three males with the live weight closest to the cage average were selected, transferred to a screened laying shed, and housed together with nine females in laying cages (32 cm wide × 38 cm deep × 16 cm high). Temperature and humidity in the sheds were monitored using thermo-hygrometers placed at bird height, which recorded the minimum and maximum temperature and humidity. Temperature was kept at 38 °C during the first three days using a wood-burning stove and was decreased by 0.5 °C each day until the birds were 28 days old (Albino and Barreto, 2003). The photoperiod was 24 h light:0 h dark (natural + artificial light with 60 W lamps) for the first 2 days of life, 23 h light:1 h dark until day 15, then 14 h light:10 h dark until the end of the growth phase. At 36 days of age, light was increased by 30 min each day up to 17 h

light:7h dark, which was retained until the breeding period ended (Faitarone et al., 2005; Barreto et al., 2007).

For both phases (growth and production), a completely randomized design was used, with five treatments and six replicates (cages). The treatments consisted of five dietary CP levels (18%, 20%, 22%, 24% and 26%) only during the growth phase, as part of feeds that were isoenergetic and isonutritious for the remaining nutrients. Diets were formulated based on the recommendations of Rostagno et al. (2011) by increasing or decreasing the recommended CP level (22% CP) by 2% or 4%. During the production phase, all birds received standard feed (18%) formulated per the recommendations for that phase (Rostagno et al., 2011). All diets were corn and soybean meal-based and formulated using the CP values obtained from their chemical analysis. Feed samples were also collected to determine CP concentrations (Table 1). Feed and water were supplied *ad libitum* throughout the entire 96-day experimental period.

Body Development and Performance

Two previously identified males per cage were individually weighed every three days until 60 days of age to determine the growth curve. The two birds' live weights were used to calculate the Gompertz curve, which was then used to determine the age at maturity (A), growth rate (B) and age or interval of maximum growth (M) (Grieser et al., 2018). The curve was calculated using Statistica 13.3, using the following model:

$$Weight = A \times e^{(-e^{(-B \times (M-day))})}$$

where A is the body weight at maturity, B is the growth rate, and M is the age of maximum growth (inflexion point).

The birds were weighed at 14 and 35 days old to determine the weight gain for those periods. The supplied feed and leftovers were also weighed to determine intake. Feed conversion was calculated as the intake:weight gain ratio per period.

Nitrogen Balance

At 33, 34 and 35 days of age, the total excreta were collected once daily to evaluate the nitrogen balance at the end of the growth phase. At the beginning and end of the collection period, the feed was weighed to determine intake. Collection trays were inserted under each cage and lined with resistant plastic to avoid contamination and loss of excreta. All collections began at 08:00. Feathers and feed particles were removed, and excreta were placed in plastic bags and stored at -20 °C until the end of the collection period. The excreta were then thawed, homogenized and weighed. Aliquots of 300 g were removed and predried in a forced air circulation oven (55 °C) for 72 hours. The samples were again weighed to determine the predry matter, then ground using a knife mill equipped with a 2-mm sieve. Excreta and feed samples were analyzed to determine dry matter and nitrogen concentrations (AOAC, 2005). Nitrogen intake and excreta were quantified based on the results, and the percent nitrogen retained was calculated.

Testicular Development

At 35 and 60 days of age, two males from each experimental unit were selected based on their average weight, euthanized by cervical dislocation and exsanguination, and dissected for testicular harvesting. The right and left testis were removed, weighed separately, and measured (width × thickness × height) using a digital pachymeter (Digimess, São Paulo, Brazil). Testicular weight was calculated as the sum of the weight of the right and left testis. Testicular volume was calculated using the equation: $V = 4 \div 3\pi ab^2$, where a is half the

testis height, and *b* is half the testis width (Yadav and Chaturvedi, 2015). The gonadosomatic index, which is the percentage of body weight corresponding to the gonads, was estimated using the equation, $GI = \left(WTl + {WTr}/{LW}\right) \times 100$, where *WTl* is the left testis weight, *WTr* is the right testis weight, and LW is the live weight. After measuring these parameters, the testes were fixed in Bouin's solution for approximately 12 hours at ambient temperature, then washed in 70% alcohol for subsequent histological analysis (Khalil et al., 1989).

Histological Analysis

The testes were histologically analyzed at the Laboratory of Histology and Immunohistochemistry of the Department of Animal Science of the UFLA. First, the testes were dehydrated in a graded ethanol-xylol series and embedded in paraffin. Sections of 5 µm thick were obtained, suspended in a water bath at approximately 37 °C, mounted on silanized histological slides, and dried in an oven at 37 °C overnight. The samples were then deparaffinized, rehydrated in a xylol-ethanol series, and stained with hematoxylin-eosin (Suvarna et al., 2018).

Seminiferous tubule images were analyzed at 400× magnification using an Olympus CX31 microscope (Olympus, Tokyo, Japan) coupled to a digital Altra SC30 camera (Olympus, Tokyo, Japan) using Axio Vision software (Carl Zeiss, Oberkochen, Germany). In each section, the highest and lowest diameters of 10 random round-shaped tubules were measured to calculate the seminiferous tubular area, using the equation $A = \pi r^2$, where r is the tubule radius calculated from the average highest and lowest diameter. The seminiferous epithelial height was obtained by performing seven measurements per tubule and analyzed to calculate the area.

Immunohistochemistry

Immunohistochemical analyses were performed in the Laboratory of Histology and Immunohistochemistry of the Department of Animal Science of UFLA. Slides were prepared as described for the histological analyses per Suvarna et al. (2018) with some adaptations. All slides were incubated in a humidified chamber, and all washes consisted of three consecutive immersions for five minutes in 0.1 M phosphate-buffered saline (PBS) at pH 7.2. Endogenous peroxidase activity was blocked by incubation in Peroxidase Block (K4011, DakoCytomation, USA) for 30 minutes and washed in PBS. Nonspecific antibody binding was blocked by incubating the sections with Block Serum (X0909, DakoCytomation, USA) for 15 minutes. The histological sections were then washed again in PBS, incubated for 2 h with mouse anti-PCNA monoclonal primary antibody (M0879, DakoCytomation, USA) (Reitemeier et al., 2013), washed in PBS, incubated for 15 minutes in Biotinylated Link Universal (K0690 DakoCytomation, USA), washed in PBS for 5 minutes, and incubated in Streptavidin-HRP (K0690 DakoCytomation, USA) for 15 minutes per the recommendations from the anti-rabbit and anti-mouse secondary antibody kit LSAB + System/HRP (K0690, DakoCytomation, USA). After washing, the histological sections were developed enzymatically using 3,3-diaminobenzidine tetrahydrochloride (DAB) (DakoCytomation, USA) and immersed in distilled water after 40 seconds to stop the reaction (samples from birds aged 35 days) or after 1 minute (samples from birds aged 60 days). The slides were then counterstained with hematoxylin and mounted under a coverslip.

The number of Sertoli cells per tubule and the percentage of proliferating spermatogonia were determined in 10 tubules section per testis and observed at 400× magnification using a light microscope (Olympus CX31, Olympus, Tokyo, Japan). The Sertoli cell number per area (10000 μm^2) was calculated using the tubular area. The number

and percentage of proliferating spermatogonia was determined by counting the stained cells relative to the total number of cells located closer to the basal membrane (Figure 1).

The number of Leydig cells and the tubular and intertubular areas were evaluated at 1000× (35 days of age) of 400× (60 days of age) using an Olympus CX31 microscope (Olympus, Tokyo, Japan) coupled to the Altra SC30 digital camera (Olympus, Tokyo, Japan) in an Axio Vision software (Carl Zeiss, Oberkochen, Germany). Ten fields randomly distributed in the testicular parenchyma were evaluated on a grid projected through the ImageJ version 1.50i program (NIH, USA). One hundred two intersection points were considered. The Leydig cell number per area (10000 μm^2) was calculated using the proportion of the leydig cells per intertubular area and the intertubular area.

Fertility Test

On day 60 of the experiment, all eggs were collected from each cage. Eggs that were cracked, broken, dirty, too big or too small were discarded, and the remainder were labeled and stored for 24 hours at 20 °C. The eggs were then weighed, and ten eggs were selected per cage based on average weight. The selected eggs were sterilized with a 2:1 formaldehyde (37%):potassium permanganate (99%) solution (Oznurlu et al., 2016) and incubated at 37.5 °C and 60% humidity (Ben-Ezra and Burness, 2017) in an automatic incubator (Luna 480, Chocmaster, Piraquara, Brazil). The automatic egg-turning tray turned the eggs every 2 hours until day 15 of incubation (Bhagat et al., 2012). After 21 days of incubation, the number of hatched and fertilized but unhatched eggs were counted, and percent fertility was calculated.

Semen Quality

Semen quality was evaluated from one male per cage. From day 50 of the experiment, semen was collected every two days via dorsal-abdominal massage.

194 Semen was evaluated with or without the presence of foam (Biswas et al., 2013).
195 Foam was collected from each male's cloacal gland at days 80, 81 and 82 of the experiment at
196 08:00 and 16:00 each day, by gently squeezing the cloacal gland on each side. After
197 collection, the foam was stored in Falcon tubes at -20 °C. At the end of the collection period,
198 the samples obtained on the previous days were thawed at ambient temperature and diluted
199 1:4 (foam:saline) in saline solution (0.9%). The samples were then centrifuged at 3000 g for
200 45 min (Sorvall™ ST 16 Centrifuge, Thermo Fisher Scientific, Massachusetts, USA). The
201 supernatant was collected and stored at -80 °C until semen quality analysis.

202 At 90 days of age, the birds' semen was collected three times at 3-day intervals per
203 Burrows and Quinn (1937). Before collection, the feathers in the pericloacal region were
204 removed, and the size of the cloacal gland was measured (width × thickness × height) using a
205 digital pachymeter (Digimess, São Paulo, Brazil) to calculate the cloacal gland area (GA),
206 using the equation $GA = W \times H$, where W is the lateral width, and H is the dorsoventral
207 height (Fields et al., 1979).

208 Foam was removed from the cloacal gland during the semen collection. The birds
209 were massaged dorsally, beginning near the wing base and ending near the cloaca, with six
210 movements per animal as standard. Slight pressure was then applied with the fingers on the
211 base of the phallus and on the ampoules of the vas deferens for semen discharge. The seminal
212 content was collected in graduated capillary tubes, where its volume was measured, and
213 immediately diluted at 1:1 (semen:saline) in saline solution (0.9%). A 1 ml aliquot of solution
214 was diluted to 499 ml of formalin for subsequent sperm concentration analysis which was
215 performed using the Neubauer chamber. Another 1 ml aliquot was diluted to 98 ml of 0.9%
216 saline solution or 0.9% saline solution + 5% foam (Biswas et al., 2010).

217 Immediately after dilution in saline solution or saline solution + foam, three trained
218 evaluators blindly evaluated the sperm motility and movement intensity from three

subsamples mounted between glass slides and coverslips at 37 °C and observed at 200× magnification using a light microscope (Olympus CX31, Olympus, Tokyo, Japan). Sperm motility was expressed as the percentage of motile spermatozoa, and movement intensity was classified from 0 to 5, with 0 being the lowest and 5 the highest.

Sperm viability was evaluated by mixing one drop of semen with one drop of eosin-nigrosin (Blom, 1950) on glass slides and observing the mixture at 400× magnification using a light microscope (Olympus CX31, Olympus, Tokyo, Japan). The numbers of living (no color) and dead (pink) cells were counted, and sperm viability was calculated as living cells/total number of cells × 100.

Statistical Analysis

The Gompertz curve parameters (A, B and M) obtained for each experimental unit and the male reproductive performance and quality data were checked for normality (Anderson-Darling), homoscedasticity (Breusch-Pagan) and independence of error (Durbin-Watson). When the assumptions of normality were met, an analysis of variance (ANOVA) was performed, and a regression analysis was performed for the protein levels. When a linear regression could not be fit (R^2<0.70), a broken-line analysis was performed to determine the best protein level (Wen et al., 2016). When a curve could not be fit by broken-line analysis, the averages were compared using the Student-Newman-Keuls (SNK) test at $P \leq 0.05$. When the ANOVA assumptions were unmet, and the Box-Cox and Johnson data transformations could not normalize the data, the data were subjected to a nonparametric analysis, and the averages were compared using the Kruskal-Wallis test. All statistical analyses were performed using Statistica 13.3 and Action 3.5 software.

RESULTS

Growth Characteristics and Performance

The different dietary CP levels in the growth diets resulted in different growth patterns in male quails (Table 2, Figure 2). The growth rate (maturity rate) increased linearly ($P < 0.01$), and the age of maximum growth (curve inflexion point) decreased ($P < 0.05$) with increasing dietary CP up to 22% CP (Figure 3). No effect was observed on body weight at maturity ($P > 0.05$).

Live weight was affected from the second until the seventh week of the birds' lives (Table 3). Until 36 days of age, 23% CP or higher resulted in greater live weights (Figure 4). To obtain greater weights at 42 and 48 days of age, 23.5% CP is recommended.

Diets with 24% and 26% CP resulted in more weight gain and improved feed conversion until 14 days of age ($P < 0.01$) (Table 4). Feed intake was lower, with 18% CP ($P < 0.01$). Considering the whole growth phase (1 to 35 days), diets with 22% CP or more resulted in less weight gain ($P < 0.01$). No effect on feed conversion was observed in this phase ($P > 0.05$). At the end of the growth phase (33 to 35 days of age), both nitrogen intake and nitrogen excretion increased linearly with increasing dietary CP ($P < 0.01$). No differences were observed in nitrogen retention ($P > 0.05$).

Reproductive Characteristics

At 35 days of age, anatomical and histological evaluations of the quail testes were affected by the dietary CP level ($P < 0.01$) (Table 5). The size of the right and left testes and seminiferous tubular areas increased linearly ($P < 0.01$) with increasing dietary CP levels (Figure 5). Germinal epithelial height increased ($P < 0.01$) only up to 22% CP (Figure 6). Higher intertubule:tubule ratio was osberved ($P < 0.01$) with 24 and 26% CP. There was

linear effect ($P < 0.01$) of dietary CP in number of spermatogonia and Leydig cells in the testis.

At 60 days of age, linear increase ($P < 0.01$) in Sertoli cell numbers was observed with increasing CP levels of the diet. Higher Leydig cell numbers was observed ($P < 0.01$) with 18% CP and more spermatogonia/area with 24 and 26% CP ($P<0.05$).

At 90 days of age, the dietary CP levels in the growth diet did not affect cloacal gland size, foam weight and protein concentration, semen volume and quality, or flock fertility ($P > 0.05$) (Table 6).

DISCUSSION

Although dietary CP level affected body development and Sertoli cell numbers and the number of spermatogonia in male Japanese quails, their reproductive efficiency was unaffected during the production phase. These results suggest that the lowest CP level tested (18% CP) or the recommended CP level for females (24%) (NRC, 1994; Rostagno et al., 2017) can be used in breeder flocks.

Male fertility is essential to one-day-old chick production systems and is directly related to spermatogenesis (Lotfi et al., 2018). Spermatozoid production, in turn, depends on the Sertoli cell number and activity and the number of spermatogonia in proliferation (Alves et al., 2013). In the present study, the increased Sertoli cell numbers and the number of spermatogonia in adult birds in response to increased dietary CP during the growth phase did not affect flock fertility. This may have been because male Japanese quails naturally present high fertility rates (higher than 90%), even with decreased levels of dietary amino acids (Table 6). In addition, the similar sperm concentrations among birds receiving diets with different CP levels indicates that physiological mechanisms were regulating sperm production (Thurston and Korn, 2000). These authors suggest that follicle-stimulating hormone (FSH)

secretion is regulated by activin and inhibin. In males, FSH controls inhibin secretion for unknown reasons (Vanmontfort et al., 1995; Johnson and Brooks, 1996). This hypothesis of physiological regulation can be reinforced by the fact that the increase in the number of Sertoli cells and spermatogonia was not associated with the increase in the sperm concentration of the birds (Table 5).

In other production animal species, supplying diets to breeder males based on their own nutritional requirements is recommended (NRC, 1994; 1998; 2016; Rostagno et al., 2017). However, in quails, this need is unspecified in the main nutritional requirement tables (NRC, 1994; Silva et al., 2012; Rostagno et al., 2017). Most recommendations in the literature are based on unsexed birds or commercial females. Because males differ from females in their body development, their nutritional requirements may also differ. In the present study, dietary CP only affected body development and nitrogen balance at the end of the growth phase, without affecting reproductive efficiency in adulthood.

During the growth phase, rapid body development is directly related to reproductive organ development (Oliveira et al., 2002; Sarabia Fragoso et al., 2013). Thus, the supply of amino acids during this phase may affect bird growth. In the present study, an increase of up to 24% in dietary CP accelerated body development and led to earlier maturity. This rapid development resulted in an increased testicular size and gonadosomatic index at the end of the growth phase (35 days of age). The reduction of Leydig cell concentration per testicular unit observed with increased CP in the diet suggests that a testicular enlargement is not related to the number of Leydig cells, only to the number of tubular cells (spermatogonia and testis). This hypothesis is reinforced by the increase in the tubule:intertubule ratio stimulated by the increase in dietary CP. This could be related to an earlier reproductive age in males, which was unconfirmed in the present study. In females, the increased body weight resulting from increased dietary CP also resulted in faster development of sex organs, decreasing the age of

317 onset of sexual activity (Renema et al., 1999). Therefore, using similar diets (with 24% CP)
318 for males and females during the growth phase may benefit the reproductive system because it
319 may lower the age of onset of reproductive activities. In addition, using similar diets for both
320 males and females correlates with a greater ease of bird handling.

321 Overall, the amino acid supply should first meet the maintenance requirements, then
322 the body development and reproductive activity requirements (Wei et al., 2011). In birds,
323 excess amino acids are deaminated, and excess nitrogen is mainly eliminated as uric acid
324 (Perry et al., 2002). In Japanese quails, bodily differences between males and females are
325 evident only from 35 days of age (Retes et al., unpublished data) when the production phase
326 begins (NRC, 1994; Rostagno et al., 2017). However, growth decelerates (age of maximum
327 growth) in males at approximately 13 days of age, whereas for females it decelerates at
328 approximately 23 days of age with diets containing 24% CP, as recommended by the National
329 Research Council (NRC (1994) (Retes et al., unpublished data). In the present study, growth
330 decelerated between 13 and 14 days of age with dietary CP levels between 22 and 26%. These
331 results indicate that phased nutritional management during bird development may be more
332 effective. Rostagno et al. (2017) recommended that nutrition in Japanese quails be managed in
333 two phases: chicks (1 to 14 days) and growers (15 to 35 days). Because males present lower
334 growth rates than females, especially from 15 to 35 days of age, the nutritional requirements
335 should be overestimated for males. This explains the increased nitrogen excretion resulting
336 from increased dietary CP intake (Table 4). The absence of significant differences in nitrogen
337 retention indicates that the amino acid supply in diets with 18% CP met the birds'
338 maintenance and growth requirements at the end of the growth phase. Live weight gain during
339 the last week of growth (27 to 35 days of age) was similar among diets with different CP
340 levels (13 to 15 grams).

In the present study, the live weights of 15-day-old males increased linearly with increasing dietary CP, reaching the optimal point at 23.9% CP (Figure 4). This is consistent with the performance data for this phase (1 to 14 days), which showed that diets with 24 and 26% CP resulted in more bird weight gained and lower feed conversion (Table 4). In this case, diets with lower CP levels may have limited the bird growth because of their lower amino acid supplies. Karaalp (2009) suggested that all essential amino acid levels are sufficient in the corn- and soybean meal-based diets with 24% CP recommended for Japanese quails in the growth phase by the NRC (1994).

The protein requirements for bird body weight gain decreased until 36 days of age, going from 24% over the first two weeks to 20% at the end of the growth phase (Figure 4), which was also observed by Karaalp (2009) and Wen et al. (2016). In addition, weight gain at the end of the growth phase (1 to 35 days) did not differ between birds receiving diets with 22%, 24% or 26% CP. This indicates that phased feeding is ideal for quails during the growth phase (Karaalp, 2009; Rostagno et al., 2017) since dietary manipulation may be important in decreasing nitrogen content in the excreta and ameliorating environmental emissions and respiratory problems in sheds (Alagawany et al., 2014). The current recommendations proposed by Rostagno et al. (2017) are similar to the present results.

Improved knowledge is also needed of CP requirements for different animal species and categories by applying the concept of ideal protein to dietary formulation to optimize amino acid use. A decrease of greater than three or four percent in dietary CP, even if it meets all the amino acid requirements, may result in low productive performance (Jariyahatthakij et al., 2018). Thus, determining the true dietary CP requirements for male Japanese quails is essential to correctly apply the optimal protein concept to breeder flocks.

In the present study, levels of 23% CP or higher accelerated bodily and reproductive organ development during the growth phase, affecting the testis up to reproductive age.

However, requirements for the chick and grower phases may differ, and further studies are needed to determine the viability of using specific formulations for each rearing phase. In addition, no need for specific dietary formulations in males was evident in the present study.

CONCLUSION

Dietary CP concentration affects bodily and testicular development in male Japanese quails without affecting their reproductive efficiency. A dietary level of 23% CP is recommended for greater body development.

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511 Table 1: Calculated percentage compositions and nutrient levels in Japanese quail feed during
512 the growth (1 to 35 days) and production (36 to 96 days) phases.

	Crude protein (%)					
	18	20	22	24	26	Production
Corn	56.30	52.40	48.55	44.70	40.80	53.00
Soybean meal 45%	28.80	33.89	38.95	44.03	49.10	29.43
Wheat meal	2.00	2.00	2.00	2.00	2.00	3.00
Bicalcium phosphate	1.240	1.192	1.145	1.098	1.050	1.06
Calcitic lime	1.35	1.35	1.35	1.35	1.35	6.785
Vegetable oil	3.50	3.58	3.65	3.73	3.80	2.75
Common salt	0.399	0.399	0.398	0.397	0.397	0.322
Vitamin supplement[1]	0.100	0.100	0.100	0.100	0.100	0.100
Mineral supplement[2]	0.100	0.100	0.100	0.100	0.100	0.100
DL-methionine (99%)	0.005	0.038	0.070	0.103	0.136	0.604
L-lysine (78%)	0.052	0.039	0.026	0.013	0.000	0.358
L-threonine (99%)	0.002	0.015	0.031	0.046	0.060	0.038
L-triptophan (98%)	0.004	0.003	0.002	0.001	0.000	0.016
Choline chloride (60%)	0.050	0.050	0.050	0.050	0.050	0.037
Kaolin	6.00	4.75	3.50	2.25	1.00	2.30
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutritional composition						
Metabolizable energy (kcal/kg)	2,900	2,900	2,900	2,900	2,900	2,800
Crude protein analyzed (%)	18.32	20.09	21.92	24.14	26.39	18.04
Calcium (%)	0.900	0.900	0.900	0.900	0.900	2.909
Available phosphorus (%)	0.333	0.333	0.333	0.333	0.333	0.303
Sodium (%)	0.176	0.176	0.176	0.176	0.176	0.145
Amino acid/lisine						
Lisine (%)	100	100	100	100	100	100
Methionine+Cystine (%)	56	56	56	56	56	82
Threonine (%)	69	69	69	69	69	60
Triptophan (%)	23	23	23	23	23	21

513 [1] Content per kg of feed (minimum for all elements): 10 mg of copper, 50 mg of iron, 1.2 mg of iodine, 80 mg of
514 manganese, 0.28 mg of selenium and 60 mg of zinc.
515 [2] Content per kg of feed: 0.8 mg of folic acid, 35 mg of pantothenic acid, 1.0 mg of biotin, 40 mg of niacin,
516 11,500 IU of vitamin A, 3.0 mg of vitamin B1, 22 IU of vitamin E, 0.6 mg of vitamin B12, 4.4 mg of vitamin
517 B2, 10.0 mg of vitamin B6, 2,100 UI of vitamin D3, 1.5 mg of vitamin K3, and 125 mg de antioxidant.

518 Table 2: Growth characteristics obtained from the Gompertz curve for male Japanese quails
519 between 1 and 60 days old, receiving diets with different crude protein levels during the
520 growth phase.

Variable	Crude protein (%)					SEM	P =
	18	20	22	24	26		
Body weight at maturity (g)	149	147	146	141	141	0.42	0.55
Maturity rate	0.057*	0.069	0.078	0.089	0.091	0.01	<0.01
Age at maximum growth (days)	25.0**	16.0	14.1	12.6	12.7	0.35	0.02

521 SEM: standard error of the mean

522 * Linear effect (P<0.01)

523 ** Linear response plateau (P<0.05)

524 Table 3: Live weight of male Japanese quails receiving diets with different crude protein
525 levels during the growth phase.

Age	Crude protein (%)					SEM	P =		
(days)	18	20	22	24	26		Protein	Day	Prot*Day
6	16.3	19.5	22.9	22.7	24.6	3.16	<0.01	<0.01	<0.01
15	40.5*	52.2	57.1	63.7	61.6				
21	58.8*	74.6	79.7	86.1	87.6				
27	77.2*	96.4	100.6	106.1	107.2				
36	91.4*	111.1	113.8	119.9	122.0				
42	112.4**	124.9	131.7	134.4	130.2				
48	125.9**	131.3	136.7	137.6	133.3				
54	132.5	134.7	137.9	135.0	135.1				
60	134.2	135.9	140.4	135.4	136.5				

526 SEM: standard error of the mean

527 * Linear response plateau (P<0.01)

528 ** Quadratic effect (P<0.01)

529 Table 4: Performance and nitrogen balance of male Japanese quails receiving diets with
530 different crude protein levels during the growth phase.

Variable	Crude protein (%)					SEM	P =
	18	20	22	24	26		
1 to 14 days							
Weight gain (g)	24.8 a	35.2 b	38.6 b	46.2 c	45.6 c	0.13	<0.01
Feed intake (g)	65.1 a	86.2 b	88.6 b	89.4 b	94.7 b	3.78	<0.01
Feed conversion	2.65 a	2.45 ab	2.29 b	1.96 c	2.07 c	0.11	<0.01
1 to 35 days							
Weight gain (g)	84 a	102 b	108 c	110 c	111 c	0.26	<0.01
Feed intake (g)	289 a	335 b	350 b	353 b	359 b	6.68	<0.01
Feed conversion	3.46	3.29	3.25	3.22	3.25	0.06	0.07
Nitrogen balance at 35 days							
N ingested (mg/bird/day)	0.41*	0.49	0.54	0.59	0.66	0.01	<0.01
N excreted (mg/bird/day)	0.19*	0.25	0.30	0.34	0.40	0.01	<0.01
N retained (mg/bird/day)	0.22	0.23	0.25	0.25	0.26	0.01	0.32

531 SEM: standard error of the mean

532 [a,b] Means followed by different letters within the same row differ significantly by the Student-Newman-Keuls
533 test (P<0.05)

534 * Linear effect (P<0.05)

535 Table 5: Growth characteristics and histological analyses of the testes of male Japanese quails
536 aged 35 and 60 days receiving diets with different crude protein levels during the growth
537 phase.

Variable	Crude protein (%)					SEM	P =
	18	20	22	24	26		
			- 35 days -				
Gonadosomatic index	0.53*	0.45	0.98	1.38	1.53	0.11	<0.01
Right testis							
Weight (g)	0.24*	0.24	0.52	0.76	0.80	0.05	<0.01
Height (mm)	8.27*	9.60	13.33	14.79	14.99	0.85	<0.01
Width (mm)	5.06*	5.65	7.58	8.58	9.05	0.52	<0.01
Thickness (mm)	4.88*	5.41	7.08	8.30	8.58	0.48	<0.01
Volume (cm³)	0.11 *	0.20	0.41	0.60	0.66	0.06	<0.01
Left testis							
Weight (g)	0.24*	0.24	0.56	0.76	0.94	0.06	<0.01
Height (mm)	7.70*	8.88	12.54	13.62	14.13	0.78	<0.01
Width (mm)	5.56*	6.03	8.44	9.44	10.35	0.61	<0.01
Thickness (mm)	5.50*	5.87	8.15	9.02	9.85	0.59	<0.01
Volume (cm³)	0.13*	0.21	0.50	0.66	0.82	0.06	<0.01
Seminiferous tubular area (µm²)	13314*	15806	28986	33727	34927	3289	<0.01
Germinal epithelial height (µm)	42.6**	46.7	63.0	63.6	65.8	4.18	<0.01
Tubule:intertubule ratio	11.3 a	17.2 b	17.9 b	21.1 c	20.6 c	2.60	<0.01
Leydig cells/area (10.000 µm²)	10.7*	10.0	7.3	5.7	5.1	0.57	<0.01
Leydig cells/tubule	36.9*	34.4	25.0	19.5	18.9	0.34	<0.01
Sertoli cells/area (10.000 µm²)	2.47	2.44	2.60	2.21	2.45	0.34	0.95
Sertoli cells/tubule	22.6	19.1	19.2	15.6	16.5	1.84	0.10
Spermatogonia / area (10.000 µm²)	3.69*	4.34	7.88	8.41	9.55	0.80	<0.01
Spermatogonia / tubule	27.1*	33.0	54.8	62.0	63.7	0.31	<0.01
% proliferating spermatogonia	74.7*	83.1	88.4	95.9	97.2	0.34	0.01
			- 60 days -				
Gonadosomatic index	4.39	4.03	3.67	4.01	3.87	0.17	0.15
Right testis							
Weight (g)	2.73	2.51	2.61	2.49	2.41	0.08	0.15
Height (mm)	23.05	23.63	23.3	22.98	22.41	0.56	0.75
Width (mm)	14.27	13.96	13.81	13.8	13.53	0.30	0.66

Thickness (mm)	13.58	13.00	13.29	13.27	12.85	0.18	0.16
Volume (cm³)	2.36	2.25	2.09	2.21	2.06	0.12	0.37
Left testis							
Weight (g)	2.86	2.54	2.7	2.57	2.66	0.03	0.54
Height (mm)	21.67	21.17	20.67	20.73	21.39	1.09	0.59
Width (mm)	15.08	14.72	15.24	14.88	14.92	0.31	0.87
Thickness (mm)	14.77	14.31	14.55	14.48	14.49	0.33	0.94
Volume (cm³)	2.55	2.35	2.42	2.36	2.43	0.15	0.89
Seminiferous tubular area (µm²)	73477	75312	69823	72781	67841	3576	0.60
Germinal epithelial height (µm)	86.9	89.4	87.9	88.5	84.1	2.82	0.72
Tubule:intertubule ratio	33.1	32.9	42.9	33.6	41.5	6.13	0.63
Leydig cells/area (10.000 µm²)	24.8 a	14.4 b	13.1 b	11.5 b	11.8 b	3.12	<0.01
Leydig cells/tubule	13.6 a	7.9 b	7.0 b	6.3 b	6.5 b	1.71	<0.01
Sertoli cells/area (10.000 µm²)	1.16*	1.23	1.32	1.43	1.58	0.05	<0.01
Sertoli cells/tubule	8.25*	8.51	9.66	10.28	10.67	0.47	<0.01
Spermatogonia / area (10.000 µm²)	10.9 a	10.3 a	10.9 a	11.4 b	12.7 c	0.55	0.05
Spermatogonia / tubule	80.1 a	78.3 a	76.8 a	84.0 b	84.7 b	3.95	0.05
% proliferating spermatogonia	95.5	95.3	96.1	94.1	94.9	0.65	0.31

538 SEM: standard error of the mean

539 * Linear effect (P<0.01)

540 ** Linear response plateau (P<0.01)

541 [a,b] Means followed by different letters within the same row differ significantly by the Student-Newman-Keuls

542 test (P<0.05)

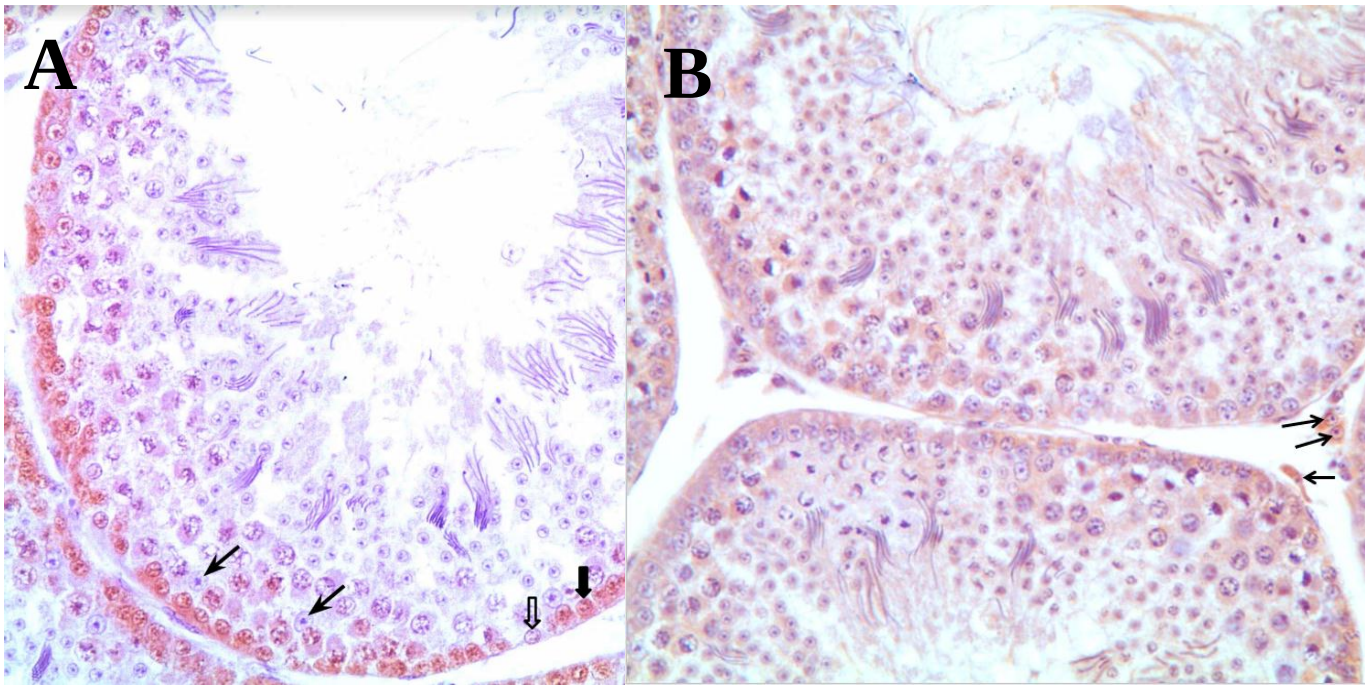
543 Table 6: Reproductive performance of male Japanese quails aged 90 days receiving diets with
544 different crude protein levels during the growth phase.

Variable	Crude protein (%)					SEM	P=
	18	20	22	24	26		
Cloacal gland	13.6	13.9	14.1	14.2	14.1	0.24	0.39
Height (mm)	20.1	20.8	20.4	20.5	21.2	0.40	0.35
Width (mm)	2.73	2.90	2.86	2.91	3.00	0.10	0.38
Area (cm^2)	0.14	0.16	0.14	0.12	0.11	0.07*	0.67
Foam weight (g)	1.62	1.65	1.69	1.71	1.55	0.12	0.87
Foam protein (g/dL)	3.56	3.28	2.85	3.63	3.30	0.38	0.64
Semen volume (mL)	13.6	13.9	14.1	14.2	14.1	0.24	0.39
Semen concentration ($\times 10^6$ sptz/µl)	1.41	1.43	1.30	1.45	1.68	0.20	0.75
Semen without foam							
Motility (%)	67.9	67.1	64.1	70.4	68.9	3.60	0.79
Vigor	3.14	3.07	3.03	3.35	3.06	0.15	0.59
Viability (%)	95.6	95.4	95.6	93.9	96.8	0.42	0.42
Semen with foam							
Motility (%)	68.8	67.9	64.4	67.4	68.4	4.15	0.95
Vigor	3.42	3.58	3.63	3.42	3.79	0.15	0.40
Viability (%)	95.7	86.1	96.3	94.3	95.3	0.44	0.84
Fertility (%)	91.7	89.8	94.4	95.4	90.7	-	0.56

545 SEM: standard error of the mean

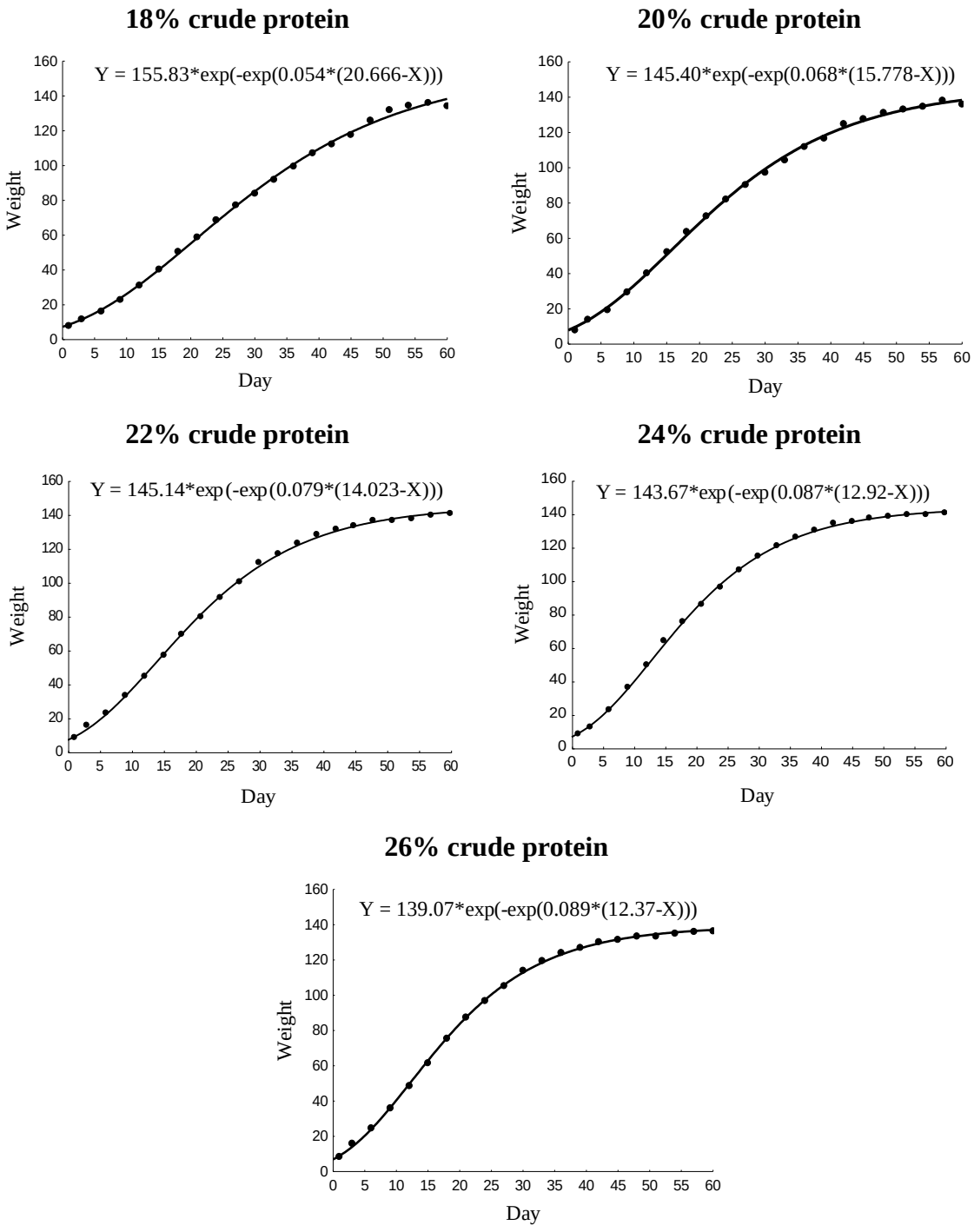
546 * Box-Cox data transformation

547

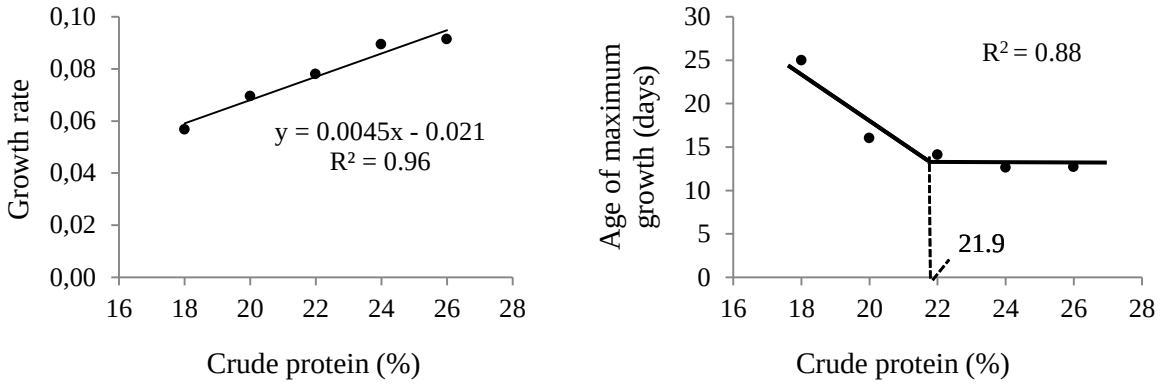


548 Figure 1. Immunohistochemical localization of spermatogonia, Sertoli and Leydig cells in
549 testicle of Japanese quail at 60 days of age. **A.** Thin arrows: Sertoli cells. Thick arrow: stained
550 spermatogonia. Unfilled arrow: unstained spermatogonia. B. Arrows: Leydig cells.
551 (magnification, ×400).

552

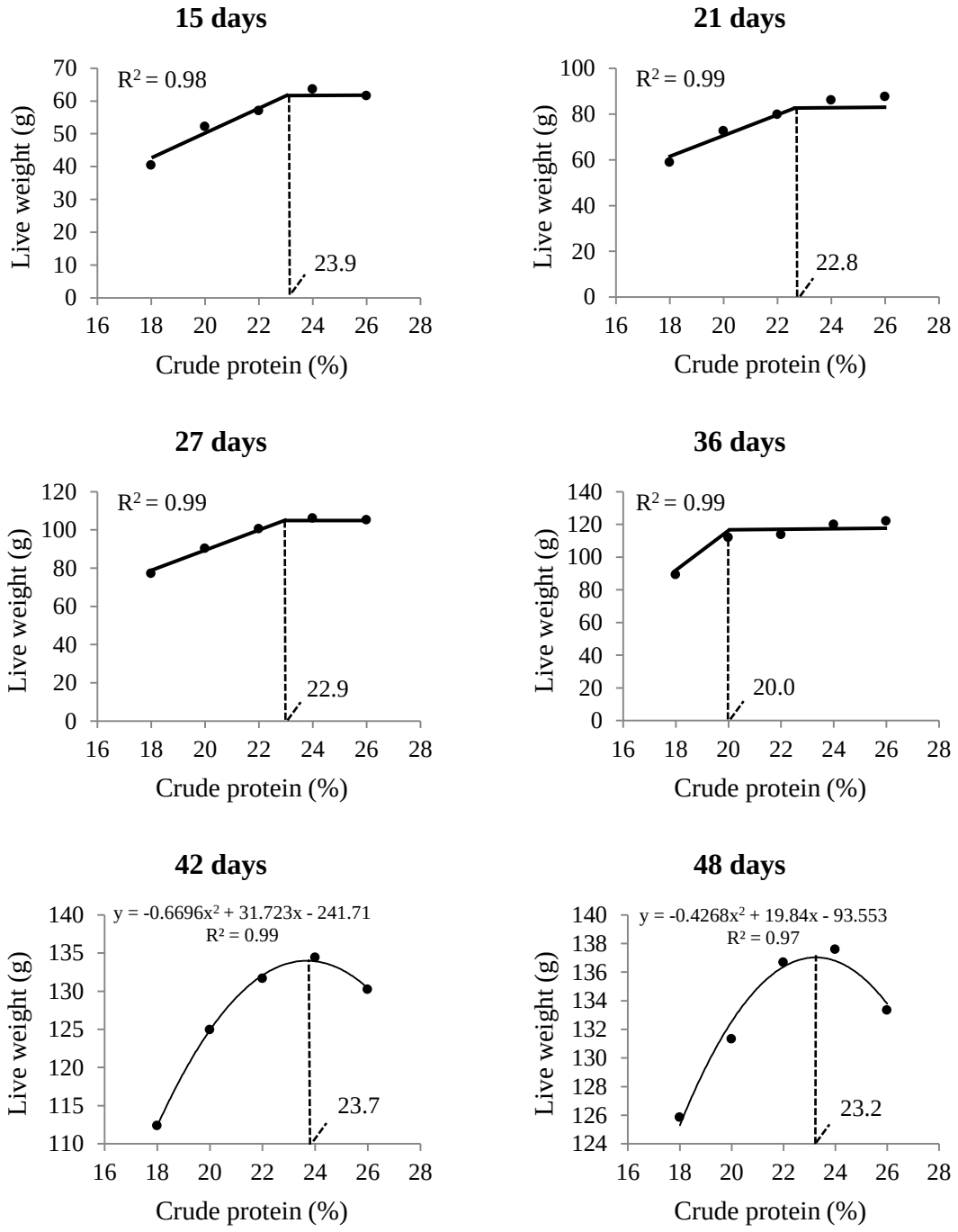


553 Figure 2. Gompertz model growth curves for male Japanese quails receiving diets with
554 different crude protein levels during the growth phase.



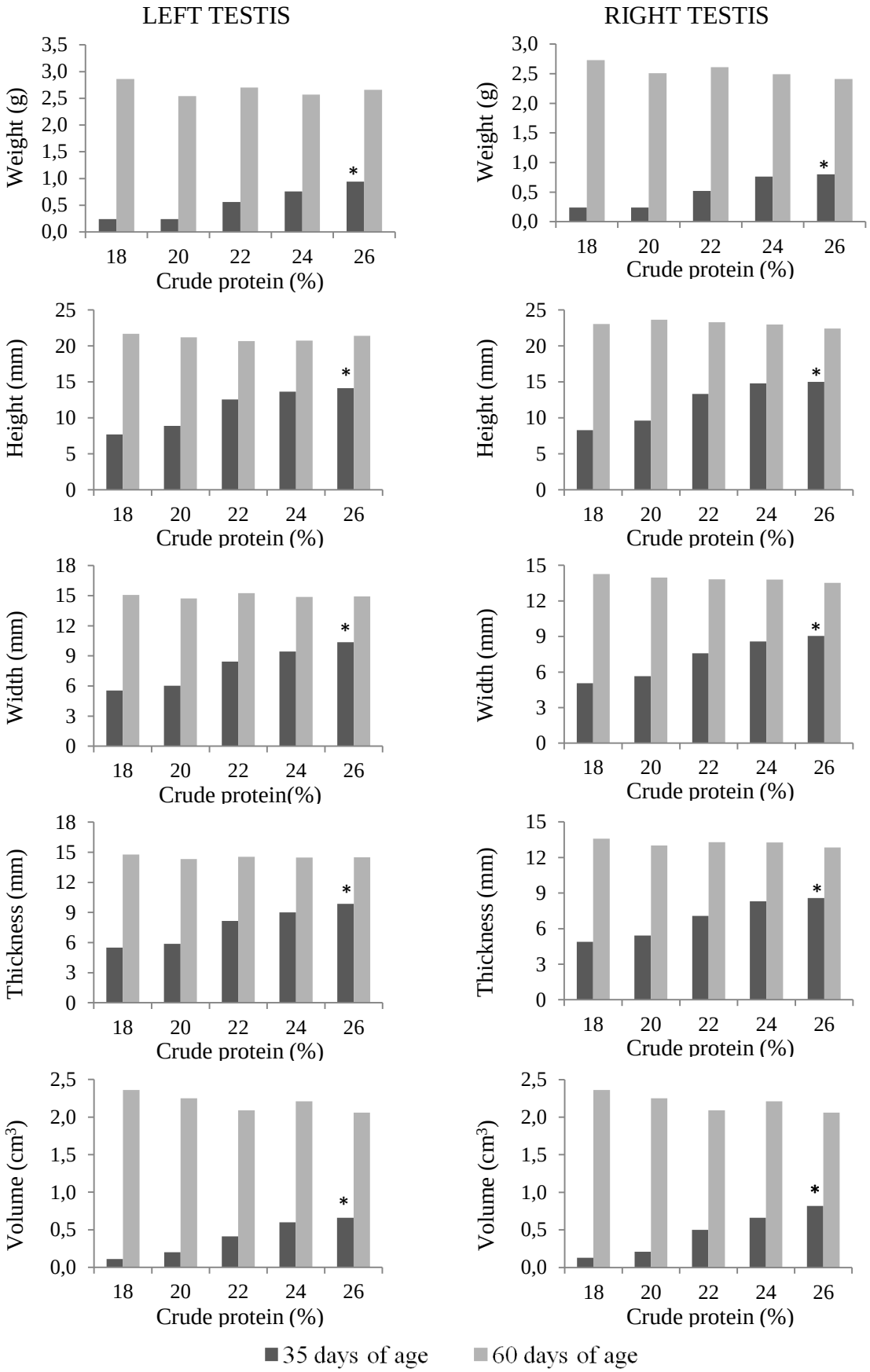
555 Figure 3: Growth characteristics obtained from the Gompertz curve for male Japanese quails
556 aged 1 to 60 days, receiving diets with different crude protein levels during the growth phase.

557

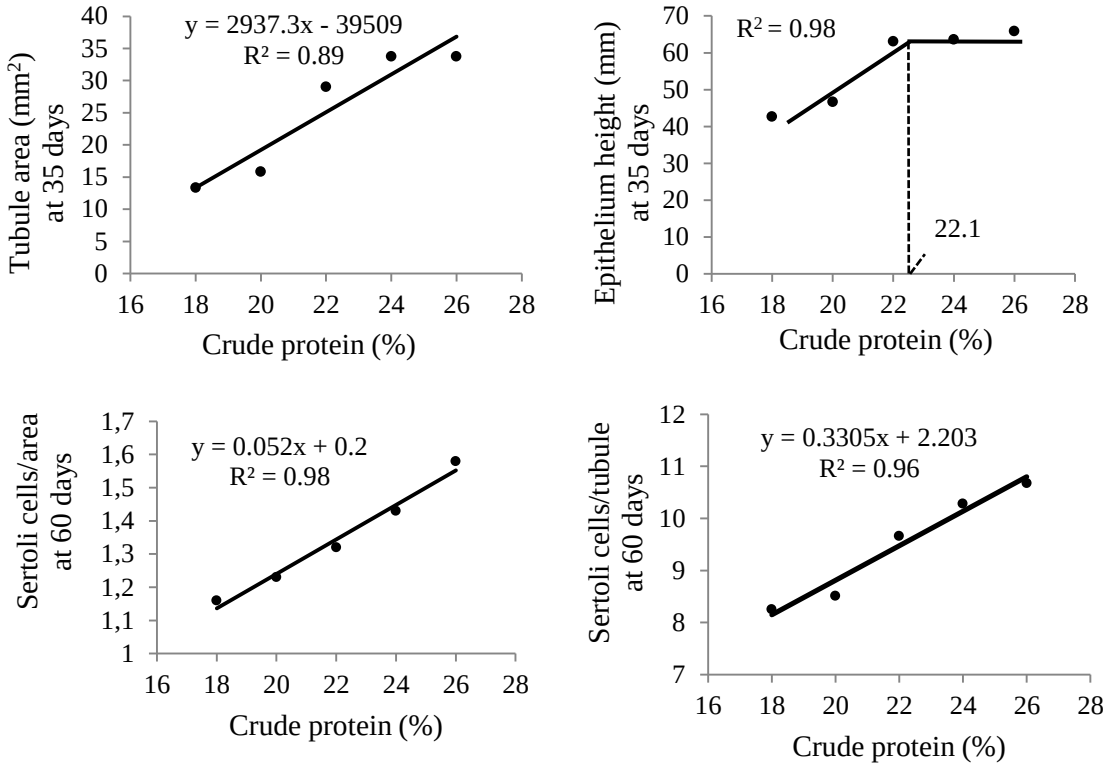


558 Figure 4. Live weight of male Japanese qualis, in diferente ages, receiving diets with different

559 crude protein levels during the growth phase.



560 Figure 5. Anatomical characteristics of the left and right testes of male Japanese quails aged
561 35 and 60 days receiving diets with different crude protein levels during the growth phase. *
562 Linear effect (P<0.01).
563



564 Figure 6. Seminiferous tubular area and testis germinal epithelial height at 35 days of age and
565 number of Sertoli cells per area and per tubule at 60 days of age of Japanese quails receiving
566 diets with different crude protein leves during the growing phase.

567

MANUSCRIPT 3

Reproductive characteristics of male Japanese quails fed diets with different levels of crude protein during the growth and production phases

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Abstract. The nutritional requirements of male reproductive quails have been poorly studied and the use of diets based on the requirements of females has been discussed. The objective of this study was to verify the influence of different dietary levels of crude protein (CP) on the reproductive characteristics of Japanese quails. Three hundred one-day-old male Japanese quail were distributed in a completely randomized design with five treatments (18, 20, 22, 24 and 26% CP levels) in six replicates of 10 males each. At 35 days, dietary CP was reduced by four percentage units (14, 16, 18, 20 and 22%). At this age, three birds from each experimental plot were transferred to the production shed and housed together with nine females to evaluate reproductive performance at 60 days of age. At the end of the experiment, two males from each experimental unit were slaughtered for anatomical and histological evaluations of their testes. The dietary CP did not influence ($P>0.05$) the physical characteristics of the testis, the number of Leydig and Sertoli cells, as well as the number of spermatogonia. There was also no difference ($P>0.05$) in the volume and concentration of semen, sperm motility, sperm viability and the fertility of the males. It is concluded that the variation of CP in the diet of male Japanese quails does not influence the reproductive characteristics of the birds.

Additional keywords: Bird, fertility, cell proliferation, semen quality.

Introduction

Quail production is an activity that is developing around the world due to its properties, such as the low initial investment, precocity of birds, good productivity, high fertility, small areas for breeding and lower labor costs (Gaviol *et al.* 2008), as well as the high nutritional value of both quail meat and eggs (Chepkemoi *et al.* 2017). However, despite the high fertility of quails, most of the reproductive problems found in breeding are related to

males. Because there are less males compared to females (Ipek *et al.* 2004), infertile males have a higher economic impact, significantly reducing the number of hatched eggs and, consequently, the economic efficiency of a breeding.

Although males have an important participation in reproductive efficiency, the nutritional requirements of this sexual category are not yet well established. Most of the nutritional requirement tables are based on the performance of non-sexed birds or females only (NRC 1994; Rostagno *et al.* 2017). However, since males and females present different growth rates and productive capacities, it is assumed that the nutritional requirements of the different sexes are also different.

Dietary crude protein (CP) represents an important source of amino acids that are used not only for maintenance, but also for growth and production (Wei *et al.* 2011). During the growth phase, the presence of amino acids is directly related to body development (Renema *et al.* 1999; Lima *et al.* 2016). At this stage, both an excess and lack of these nutrients can impair organ function in adult life (Jordão Filho *et al.* 2012).

In spite of the several studies reinforcing the importance of dietary CP in the development of the reproductive tract of female quails (Lilburn *et al.* 1992; Santos *et al.* 2012; Agboola *et al.* 2016; Ratriyanto *et al.* 2017), its importance in the development of the reproductive organs of males has received little attention. Studies indicate that body growth has a high correlation with the development of reproductive organs (Fontana *et al.* 1990; Lilburn *et al.* 1992). In this case, the use of specific diets for females could influence the reproductive performance of males, since the delay in the development of reproductive organs caused by an inadequate protein balance may influence sexual maturity and reproductive performance (Renema *et al.* 1999; Lima *et al.* 2016).

As occurs in other species (Ghonim *et al.* 2010; Shanmugam *et al.* 2016), it is probable that a protein imbalance may influence the reproductive characteristics of male

quails, causing economic losses due to the reduction in the number of fertile eggs. Studies evaluating the influence of different CP levels in the diet of male Japanese quails are scarce. As a hypothesis, it is believed that the use of diets based on the requirements of females has no influence on the reproductive performance of males. Thus, the objective of this study was to evaluate the influence of different dietary levels of CP on the reproductive characteristics of male Japanese quails.

Material and methods

Local, animals and experimental design

This experiment was performed between March and June 2018 at the Department of Animal Science of the Federal University of Lavras (UFLA) in Lavras, Minas Gerais, Brazil. The Animal Research Ethics Committee approved all experimental procedures under protocol no. 057/2017.

Three hundred male one-day-old Japanese quails (*Coturnix coturnix japonica*) were randomly housed in a masonry shed in 30 cages (50 cm wide × 70 cm deep × 25.5 cm high) until 35 days of age (growth phase). At this age, three males with the live weight closest to the cage average were selected, transferred to a screened laying shed, and housed together with nine females in laying cages (32 cm wide × 38 cm deep × 16 cm high) until 60 days of age (production phase). The temperature and humidity of the sheds were monitored using thermo-hygrometers placed at bird height, which recorded the minimum and maximum temperature and humidity. Temperature was kept at 38 °C during the first three days using a wood-burning stove and was decreased by 0.5 °C each day until the birds were 28 days old (Albino and Barreto 2003). The photoperiod was 24 h light:0 h dark (natural + artificial light with 60 W lamps) for the first 2 days of life, 23 h light:1 h dark until day 15, then 14 h light:10 h dark until the end of the growth phase. At 36 days of age, light was increased by 30 min each day

up to 17 h light:7h dark, which was retained until the breeding period ended (Faitarone *et al.* 2005; Barreto *et al.* 2007).

A completely randomized design was used, with five treatments and six replicates (cages). The number of birds per experimental unit was two or three, depending on analyzed variable. The treatments were five feed programs that consisted of five different dietary CP levels (18, 20, 22, 24, and 26%) during the growth phase, which were reduced by four percentage units (14, 16, 18, 20, and 22%) during the production phase, according to recommendations (NRC 1994; Rostagno *et al.* 2017). Diets were isoenergetic and isonutritious for the remaining nutrients (Tables 1 and 2). Nutritional requirements were met, according of Rostagno *et al.* (2011), with the exception of CP. All diets were corn and soybean meal-based and formulated using the CP values obtained from their chemical analysis. Feed samples were collected to determine CP concentrations. Feed and water were supplied *ad libitum* throughout the entire 60-day experimental period.

Testicular development

At 60 days of age, two males from each experimental unit were selected based on their average weight, euthanized by cervical dislocation and exsanguination, and dissected for testicular harvesting. The right and left testes were removed, weighed separately, and measured (width × thickness × height) using a digital pachymeter (Digimess, São Paulo, Brazil). Testicular weight was calculated as the sum of the weight of the right and left testis. Testicular volume was calculated using the equation: $V = 4 \div 3\pi ab^2$, where a is half the testis height, and b is half the testis width (Yadav and Chaturvedi 2015). The gonadosomatic index, which is the percentage of body weight corresponding to the gonads, was estimated using the equation: $GI = \left(WTl + {}^{WTr}/_{LW}\right) \times 100$, where *WTl* is the left testis weight, *WTr* is the right testis weight, and LW is the live weight. After measuring these parameters, the

testes were fixed in Bouin's solution for approximately 12 hours at ambient temperature, then washed in 70% alcohol for subsequent histological analysis (Khalil *et al.* 1989).

Histological analysis

The testes were histologically analyzed at the Laboratory of Histology and Immunohistochemistry of the Department of Animal Science of the UFLA. First, the testes were dehydrated in a graded ethanol-xylol series and embedded in paraffin. Sections (5 µm thick) were obtained, suspended in a water bath at approximately 37 °C, mounted on silanized histological slides, and dried in an oven at 37 °C overnight. The samples were then deparaffinized, rehydrated in a xylol-ethanol series, and stained with hematoxylin-eosin (Suvarna *et al.* 2018).

Seminiferous tubule images were analyzed at 400× magnification using an Olympus CX31 microscope (Olympus, Tokyo, Japan) coupled to a digital Altra SC30 camera (Olympus, Tokyo, Japan) using Axio Vision software (Carl Zeiss, Oberkochen, Germany). In each section, the highest and lowest diameters of 10 random round-shaped tubules were measured to calculate the seminiferous tubular area, using the equation $A = \pi r^2$, where r is the tubule radius calculated from the average largest and smallest diameter. The seminiferous epithelial height was obtained by performing seven measurements per tubule and analyzed to calculate the area.

Immunohistochemistry

Immunohistochemical analyses were performed in the Laboratory of Histology and Immunohistochemistry of the Department of Animal Science of UFLA. Slides were prepared as described for histological analyses, per Suvarna *et al.* (2018), with some adaptations. All slides were incubated in a humidified chamber, and all washes consisted of three consecutive

immersions for 5 minutes in 0.1 M phosphate-buffered saline (PBS) at pH 7.2. Endogenous peroxidase activity was blocked by incubation in Peroxidase Block (K4011, DakoCytomation, USA) for 30 minutes and washed in PBS. Nonspecific antibody binding was blocked by incubating the sections with Block Serum (X0909, DakoCytomation, USA) for 15 minutes. The histological sections were then washed again in PBS, incubated for 2 h with mouse anti-PCNA monoclonal primary antibody (M0879, DakoCytomation, USA; (Reitemeier *et al.* 2013), washed in PBS, incubated for 15 minutes in Biotinylated Link Universal (K0690 DakoCytomation, USA), washed in PBS for 5 minutes, and incubated in Streptavidin-HRP (K0690 DakoCytomation, USA) for 15 minutes, per the recommendations from the anti-rabbit and anti-mouse secondary antibody kit LSAB + System/HRP (K0690, DakoCytomation, USA). After washing, the histological sections were developed enzymatically using 3,3-diaminobenzidine tetrahydrochloride (DAB; DakoCytomation, USA) and immersed in distilled water after 40 seconds (samples from birds aged 35 days) or after 1 minute (samples from birds aged 60 days) to stop the reaction. The slides were then counterstained with hematoxylin and mounted under a coverslip.

The number of Sertoli cells per tubule and the percentage of proliferating spermatogonia were determined in a 10 tubule section per testis and observed at 400× magnification using a light microscope (Olympus CX31, Olympus, Tokyo, Japan). The Sertoli cell number per area (10000 μm^2) was calculated using the tubular area. The number and percentage of proliferating spermatogonia were determined by counting the stained cells relative to the total number of cells located closer to the basal membrane (Fig. 1).

The number of Leydig cells and the tubular and intertubular areas were evaluated at 400× using an Olympus CX31 microscope (Olympus, Tokyo, Japan) coupled to the Altra SC30 digital camera (Olympus, Tokyo, Japan) in an Axio Vision software (Carl Zeiss, Oberkochen, Germany). Ten fields, randomly distributed in the testicular parenchyma, were

evaluated on a grid projected through the ImageJ version 1.50i program (NIH, USA). One hundred two intersection points were considered. The Leydig cell number per area (10000 μm²) was calculated using the proportion of the Leydig cells per intertubular area and the intertubular area.

Fertility test

On days 57, 58 and 59 of the experiment, all eggs were collected from each cage. Eggs that were cracked, broken, dirty, too big or too small were discarded, and the remainder were labeled and stored for 24 hours at 20 °C. The eggs were then weighed, and 30 eggs were selected per cage based on average weight. The selected eggs were sterilized with a 2:1 formaldehyde (37%):potassium permanganate (99%) solution (Oznurlu *et al.* 2016) and incubated at 37.5 °C and 60% humidity (Ben-Ezra and Burness 2017) in an automatic incubator (Luna 480, Chocmaster, Piraquara, Brazil). The automatic egg-turning tray turned the eggs every 2 hours until day 15 of incubation (Bhagat *et al.* 2012). After 21 days of incubation, the number of hatched eggs and fertilized unhatched eggs were counted, and percent fertility was calculated.

Semen quality

Semen quality was evaluated from two males per cage that were not used for the fertility test. For the adaptation of the males, semen was collected every 2 days via dorsal-abdominal massage starting from day 49 of the experiment. Ejaculates obtained on days 58 and 60 of the experiment were evaluated.

Semen was evaluated without the presence of foam. Before collection, the feathers in the pericloacal region were removed, and the size of the cloacal gland was measured (width × thickness × height) using a digital pachymeter (Digimess, São Paulo, Brazil) to calculate the

cloacal gland area (GA), using the equation: $GA = W \times H$, where W is the lateral width, and H is the dorsoventral height (Fields *et al.* 1979). The birds were massaged dorsally, beginning near the wing base and ending near the cloaca, with six movements per animal as standard. Slight pressure was then applied with the fingers on the base of the phallus and on the ampoules of the vas deferens for semen discharge. The seminal content was collected in graduated capillary tubes, where its volume was measured, and immediately diluted at 1:1 (semen:saline) in saline solution (0.9%). A 1 mL aliquot of solution was diluted to 499 mL of formalin for subsequent sperm concentration analysis, which was performed using the Neubauer chamber. Another 1 mL aliquot was diluted to 98 mL of 0.9% saline solution (Biswas *et al.* 2010). Immediately after dilution, three trained evaluators blindly evaluated the sperm motility and movement intensity from three subsamples; these were mounted between glass slides and coverslips at 37 °C and observed at 200× magnification using a light microscope (Olympus CX31, Olympus, Tokyo, Japan).

Sperm motility was expressed as the percentage of motile spermatozoa, and movement intensity was classified from 0 to 5, with 0 being the lowest and 5 the highest. Sperm viability was evaluated by mixing one drop of semen with one drop of eosin-nigrosin (Blom 1950) on glass slides and observing the mixture at 400× magnification using a light microscope (Olympus CX31, Olympus, Tokyo, Japan). The numbers of living (no color) and dead (pink) cells were counted, and sperm viability was calculated as living cells/total number of cells × 100.

Statistical analysis

The data were checked for normality (Anderson–Darling), homoscedasticity (Breusch–Pagan) and independence of errors (Durbin–Watson). When the assumptions of normality were met, an analysis of variance (ANOVA) was performed, and a regression

analysis was performed for the protein levels. When a linear regression could not be fit (R^2<0.70), a broken-line analysis was performed to determine the best protein level (Wen *et al.* 2016). When a curve could not be fit by broken-line analysis, the averages were compared using the Student–Newman–Keuls (SNK) test at $P \leq 0.05$. When the ANOVA assumptions were not met, and the Box-Cox and Johnson data transformations could not normalize the data, the data were subjected to nonparametric analysis, and the averages were compared using the Kruskal–Wallis test. All statistical analyses were performed using Statistica 13.3 and Action 3.5 software.

Results

The dietary CP did not influence ($P>0.05$) the anatomical dimension of the cloacal gland or testis or, consequently, the gonadosomatic index (Table 3). There was also no influence ($P>0.05$) on the histological characteristics of the testis, such as the seminiferous tubular area, germinal epithelial height (μm), tubule:intertubule ratio, number of Leydig and Sertoli cells, as well as the number of spermatogonia.

An influence of dietary CP was also not observed ($P>0.05$) on the foam weight and foam protein, semen volume and concentration, sperm motility, sperm vigor and sperm viability (Tabel 4). Consequently, no difference was observed ($P>0.05$) in the fertility of the males.

Discussion

Although males play an important role within the reproductive system of quails, specific nutritional requirements for this sex category have been scarcely discussed in the literature. In the present study, it was verified that the variation of dietary CP levels did not affect the reproductive indices of males. This information is of great importance, since

nutritionists can establish nutritional programs based on specific protein requirements for females, without affecting the production of fertile eggs for breeding.

The main function of dietary CP is the supply of essential and non-essential amino acids to the organism. According to the NRC (1994), dietary requirements for lysine and sulfur amino acids are the most important for breeding quails. In the present study, digestible lysine levels ranged from 0.88 to 1.35% (variation greater than 50%) in the isoenergetic growth diets and from 0.81 to 1.28% (variation close to 60%) in the production diets. Methionine + cystine levels varied from 0.50 to 0.76% (variation greater than 50%) in the growth diets and from 0.66 to 1.05% (variation close to 60%) in the production diets. Even with this difference, the amino acid content did not influence testicular development, suggesting that, for males, the protein requirement may be much lower compared to that for females, and that excess CP does not impair the reproductive function of these birds.

In other species, dietary CP has been shown to influence the development and maintenance of reproductive activities in adult life (Ghonim *et al.* 2010; Shanmugam *et al.* 2016). When deficient, dietary CP can limit the development of the testis, since the supply of amino acids becomes insufficient to meet the requirements of muscle, feather and vital organ growth (Wei *et al.* 2011). As a consequence of lower testicular development, sperm production also becomes reduced, compromising male fertility (Lanna *et al.* 2013). This is because sperm production per gram of testis is relatively constant between different species. As a consequence, males with higher testicular mass present a higher capacity for sperm production (França and Russel 1998; Lanna *et al.* 2013). In the present study, the variation of the dietary CP levels by eight percent units did not influence the development of the testis in the initial phase and, consequently, the dimensions of these organs in the adult phase. It is known that males exhibit a relatively lower growth rate, higher age at puberty and lower body weight in adult life, in relation to females (Retes et al., unpublished data). Thus, the results

obtained in the present study may be related to the low demand of amino acids for the growth and maintenance of males. The supply of amino acids may have been enough for organ development, including the testes. This would explain the absence of the effects of diets with different CP levels on the testicular development of birds.

Because of their similar testicular development, the cloacal gland dimensions and foam weight were also not influenced by dietary CP levels. The relationship between the testes and the characteristics of the accessory reproductive organs is associated with the concentration of testosterone in the organism (Ottinger and Brinkley 1979; Biswas *et al.* 2007). In the present study, there was also no effect of dietary CP on the number of Leydig cells, the main androgen producing cell (Thurston and Korn 2000). Because the reproductive characteristics were not influenced by dietary CP, it is believed that the concentration of circulating androgens was similar among experimental diets. Also, no differences were observed in the number of Sertoli cells and spermatogonia, suggesting that not only the physical characteristics, but also the functional characteristics of the testis were preserved when the CP levels were different in the diets.

Among its several functions, testosterone acts on Sertoli cells, stimulating their metabolism during the reproductive phase (Thurston and Korn 2000). The number of Sertoli cells, in turn, is directly related to the number of germline cells (Sharpe *et al.* 2003). As the function of Sertoli cells ranges from germ cell support to hormonal regulation, it is assumed that a lack of amino acids could limit the activity of spermatogonia and its transformation into spermatozoa in the seminiferous tubules (Alves *et al.* 2013). In the present study, no effect of dietary CP levels was observed on the height of the germinal epithelium of the testes or on the number of proliferating spermatogonia, suggesting that the Sertoli cell activity was not affected by the lower amino acid intake generated by diets with lower CP levels.

With respect to the foam characteristics, the foamy exudate is formed by different chemical components, especially glycoproteins, which provide energy for spermatozoa metabolism. This stimulus increases motility and improves sperm transport in the oviduct (Singh *et al.* 2011). Thus, the chemical composition of the foam is directly related to male fertility. Despite the glycoprotein nature of the foam, CP levels in the diet did not influence its chemical composition or its production. This result, together with those obtained via histological analysis, may explain the lack of semen quality results and, consequently, the fertilization capacity of the males.

Thus, manipulation of dietary CP to meet the nutritional requirements of females may be considered an economically viable alternative, without impairing the reproductive capacity of males. In addition, the reduction of dietary CP can also be considered environmentally beneficial, since it is related to the lower elimination of pollutants by birds (Djouvinov and Mihailov 2005; Alagawany *et al.* 2014).

Conclusion

The variation in CP content of the diets, from 18 to 26% in the growth phase and from 14 to 22% in the production phase, does not affect the reproductive potential of male Japanese quails.

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Table 1. Centesimal composition and calculated nutritional levels of experimental diets for Japanese quails in the growth stages (1 to 35 days).

Ingredients	Crude protein (%)				
	18	20	22	24	26
Corn	56.30	52.40	48.55	44.70	40.80
Soybean meal	28.80	33.89	38.95	44.03	49.10
Wheat bran	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.240	1.192	1.145	1.098	1.050
Limestone	1.35	1.35	1.35	1.35	1.35
Vegetal oil	3.50	3.58	3.65	3.73	3.80
Salt	0.399	0.399	0.398	0.397	0.397
Vitamin supplement[1]	0.100	0.100	0.100	0.100	0.100
Mineral supplement[2]	0.100	0.100	0.100	0.100	0.100
DL-methionine (99%)	0.005	0.038	0.070	0.103	0.136
L-lysine (78%)	0.052	0.039	0.026	0.013	0.000
L-threonine (99%)	0.002	0.015	0.031	0.046	0.060
L-tryptophan (98%)	0.004	0.003	0.002	0.001	0.000
Choline chloride (60%)	0.050	0.050	0.050	0.050	0.050
Kaolin	6.00	4.75	3.50	2.25	1.00
TOTAL	100.00	100.00	100.00	100.00	100.00
Nutritional composition calculated					
Metabolizable energy (kcal/kg)	2,900	2,900	2,900	2,900	2,900
Crude protein (%)	18.00	20.00	22.00	24.00	26.00
Crude protein analyzed (%)	18.32	20.09	21.92	24.14	26.39
Digestible lysine (%)	0.881	0.998	1.115	1.232	1.349
Digestible methionine + cystine (%)	0.500	0.566	0.630	0.696	0.761
Digestible threonine (%)	0.613	0.692	0.773	0.854	0.934
Digestible tryptophan (%)	0.202	0.229	0.256	0.282	0.309
Calcium (%)	0.900	0.900	0.900	0.900	0.900
Available phosphorus (%)	0.333	0.333	0.333	0.333	0.333
Sodium (%)	0.176	0.176	0.176	0.176	0.176
Amino acid/lysine ratio					
Lysine (%)	100	100	100	100	100
Methionine + cystine (%)	56	56	56	56	56
Threonine (%)	69	69	69	69	69
Tryptophan (%)	23	23	23	23	23

[1] Content per kg of feed (minimum for all elements): 10 mg of copper, 50 mg of iron, 1.2 mg of iodine, 80 mg of manganese, 0.28 mg of selenium and 60 mg of zinc.

[2] Content per kg of feed: 0.8 mg of folic acid, 35 mg of pantothenic acid, 1.0 mg of biotin, 40 mg of niacin, 11,500 IU of vitamin A, 3.0 mg of vitamin B1, 22 IU of vitamin E, 0.6 mg of vitamin B12, 4.4 mg of vitamin B2, 10.0 mg of vitamin B6, 2,100 UI of vitamin D3, 1.5 mg of vitamin K3, and 125 mg de antioxidant.

Table 2. Centesimal composition and calculated nutritional levels of experimental diets for Japanese quails in the production stage (36 to 60 days).

Ingredients	Crude protein (%)				
	14	16	18	20	22
Corn	61.10	57.05	53.00	48.95	44.90
Soybean meal	19.35	24.39	29.43	34.47	39.50
Wheat bran	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.153	1.107	1.06	1.014	0.967
Limestone	6.784	6.785	6.785	6.788	6.790
Vegetal oil	2.600	2.675	2.75	2.825	2.900
Salt	0.323	0.323	0.322	0.321	0.320
Vitamin supplement [1]	0.100	0.100	0.100	0.100	0.100
Mineral supplement [2]	0.100	0.100	0.100	0.100	0.100
DL-methionine (99%)	0.427	0.516	0.604	0.692	0.780
L-lysine (78%)	0.373	0.366	0.358	0.349	0.340
L-threonine (99%)	0.037	0.038	0.038	0.038	0.038
L-tryptophan (98%)	0.023	0.020	0.016	0.013	0.009
Choline chloride (60%)	0.037	0.037	0.037	0.037	0.037
Kaolin	4.50	3.45	2.30	1.20	1.00
TOTAL	100.00	100.00	100.00	100.00	100.00
Nutritional composition calculated					
Metabolizable energy (kcal/kg)	2,800	2,800	2,800	2,800	2,800
Crude protein (%)	14.71	16.71	18.71	20.71	22.71
Crude protein analyzed (%)	14.07	16.20	18.04	20.01	21.99
Digestible lysine (%)	0.809	0.927	1.058	1.162	1.279
Digestible methionine + cystine (%)	0.664	0.761	0.868	0.953	1.049
Digestible threonine (%)	0.485	0.556	0.638	0.697	0.767
Digestible tryptophan (%)	0.170	0.195	0.220	0.244	0.269
Calcium (%)	0.909	0.909	2.909	0.909	0.909
Available phosphorus (%)	0.303	0.303	0.303	0.303	0.303
Sodium (%)	0.176	0.176	0.145	0.176	0.176
Amino acid/lysine ratio					
Lysine (%)					
Methionine + cystine (%)	100	100	100	100	100
Threonine (%)	82	82	82	82	82
Tryptophan (%)	60	60	60	60	60
Metabolizable energy (kcal/kg)	21	21	21	21	21

[1] Content per kg of feed (minimum for all elements): 10 mg of copper, 50 mg of iron, 1.2 mg of iodine, 80 mg of manganese, 0.28 mg of selenium and 60 mg of zinc.

[2] Content per kg of feed: 7,300 IU of vitamin A, 2,120 IU of vitamin D3, 8,500 IU of vitamin E, 2 mg of vitamin K3, 1.1 mg of vitamin B1, 3.5 mg of vitamin B2, 2.1 mg of vitamin B6, 5.05 μg of vitamin B12, 25.5 mg of niacin, 10 mg of pantothenic acid, 100 μg of biotin, and 18 mg of antioxidant.

435 **Table 3**. Anatomical and histological characteristics of the testes from 60-day-old Japanese
436 quails fed diets with different levels of crude protein (CP) in the growing (from 1 to 35 days
437 of age: 18, 20, 22, 24 or 26% CP) and production (from 36 to 96 days of age: 14, 16, 18, 20 or
438 22% CP) phases.

Variable	Crude protein (%)					SEM	P =
	18/14	20/16	22/18	24/20	26/22		
Cloacal gland							
Height (mm)	14.1	13.6	13.6	13.6	13.2	0.31	0.34
Width (mm)	20.8	20.1	20.3	20.5	20.1	0.45	0.56
Area (cm^2)	2.92	2.74	2.77	2.80	2.65	0.11	0.33
Gonadosomatic index	3.88	4.08	3.95	3.37	4.22	0.58	0.11
Right testis							
Weight (g)	2.52	2.70	2.59	2.57	2.78	0.35	0.59
Height (cm)	2.40	2.44	2.33	2.38	2.38	0.21	0.91
Width (cm)	1.38	1.45	1.39	1.42	1.48	0.11	0.16
Thickness (cm)	1.33	1.35	1.35	1.35	1.35	0.05	0.88
Volume (cm^3)	2.29	2.53	2.30	2.40	2.50	0.25	0.71
Left testis							
Weight (g)	2.55	2.90	2.60	2.54	2.75	0.15	0.34
Height (cm)	2.06	2.24	2.15	2.18	2.15	0.44	0.57
Width (cm)	1.44	1.55	1.48	1.50	1.53	0.11	0.21
Thickness (cm)	1.48	1.50	1.44	1.43	1.49	0.11	0.43
Volume (cm^3)	2.30	2.78	2.42	2.40	2.60	0.55	0.33
Seminiferous tubular area (μm^2)	69321	67546	67612	62399	69706	2718	0.36
Germinal epithelial height (μm)	92.8	87.3	86.3	85.5	85.7	4.59	0.10
Tubule:intertubule ratio	22.1	22.7	27.5	25.7	28.0	3.39	0.64
Leydig cells/area (10.000 μm^2)	5.1	5.1	4.4	5.5	6.9	0.98	0.47
Leydig cells/intertubule (%)	9.3	9.3	8.0	10.0	12.6	1.78	0.47
Sertoli cells/area (10.000 μm^2)	1.19	1.24	1.32	1.53	1.28	0.13	0.52
Sertoli cells/tubule	8.13	9.08	8.86	10.31	9.24	0.65	0.24
Spermatogonia / area (10.000 μm^2)	11.1	12.1	12.0	12.6	11.4	0.41	0.15
Spermatogonia / tubule	80.3	80.1	77.9	85.2	83.4	3.81	0.18
% proliferating spermatogonia	92.9	92.6	92.3	92.3	92.9	0.68	0.95

439 **Table 4**. Characteristics of ejaculates of Japanese quails fed diets with different levels of
440 crude protein (CP) in the growing (from 1 to 35 days of age: 18, 20, 22, 24 or 26% CP) and
441 production (from 36 to 96 days of age: 14, 16, 18, 20 or 22% CP) phases.

Variable	Crude protein (%)					SEM	P=
	18/14	20/16	22/18	24/20	26/22		
Foam weight (g)	0.19	0.14	0.14	0.13	0.11	0.02	0.19
Foam protein (g/dL)	1.78	1.76	1.77	1.74	1.57	0.11	0.65
Semen volume (mL)	2.71	3.50	3.86	3.81	3.61	0.49	0.20
Semen concentration ($\times 10^6$ sptz/µl)	2.07	1.56	1.49	1.57	1.74	0.22	0.37
Sperm motility (%)	72.1	74.8	76.5	72.9	73.2	2.69	0.32
Sperm vigor	3.32	3.38	3.53	3.50	3.53	-	0.44
Sperm viability (%)	95.0	96.8	96.8	96.0	95.9	0.47*	0.60
Fertility (%)	93.5	92.6	91.7	91.7	92.6	-	0.98

442 SEM: standard error of the mean

443 * Box-Cox data transformation

MANUSCRIPT 4

Proteína bruta na dieta para codornas japonesas (*Coturnix coturnix japonica*)

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RESUMO. Objetivou-se avaliar a influência de diferentes níveis de proteína bruta (PB) na dieta sobre o desempenho produtivo de codornas japonesas (*Coturnix coturnix japonica*). Um total de 360 codornas japonesas fêmeas de um dia de idade (8,7 ± 0,5 g) foi alojado em grupos de 36 fêmeas/gaiola durante as fases de cria e recria. Para a fase de produção (36 a 96 dias), as aves foram transferidas para um galpão de postura e alojadas em grupos de nove fêmeas por gaiola, totalizando 40 unidades experimentais. Os tratamentos utilizados foram cinco níveis de PB (18, 20, 22, 24 e 26% na fase de cria/recria, que foram reduzidos, respectivamente para 14, 16, 18, 20 e 22% na fase de produção). O delineamento experimental foi o inteiramente casualizado com cinco tratamentos (níveis de proteína bruta) e oito repetições com nove fêmeas cada. Aos 36 dias de idade (início da fase de produção), a PB da dieta aumentou (P<0,01) o peso relativo do maior folículo e os pesos relativos do estroma, ovário e oviduto e o número de folículos no ovário. Aos 48 e aos 58 dias de idade, a PB da dieta não influenciou (P>0,05) as características anatômicas do trato reprodutivo das aves. A intensidade de postura aumentou (P<0,01) de maneira linear com a elevação do teor de PB da dieta apenas aos 48 dias de idade das aves. Não houve efeito (P>0,05) da PB sobre a intensidade de postura aos 58 dias de idade, entretanto, aumentou (P<0,05) de maneira linear o peso dos ovos tanto aos 57 quanto aos 71 e 85 dias de idade, bem como a densidade do ovo aos 57 dias. A porcentagem de casca reduziu (P<0,05) de maneira linear aos 71 e 85 dias de idade. Não houve efeito (P>0,05) sobre a unidade Haugh, nas porcentagens de gema e albúmen e nos teores de matéria seca e proteína nos principais componentes do ovo. Conclui-se que o teor de PB na dieta influencia a produção e a qualidade de ovos codornas japonesas. Para aumentar a produção de ovos até o pico de postura, recomenda-se 26% de PB na fase de crescimento e 22% na fase de produção. Para aumentar o peso dos ovos sem comprometer a produção, recomenda-se manter 22% de PB até os 90 dias de idade ou mais.

Palavras-chave: coturnicultura, desempenho, nutrição, produção de ovos, qualidade do ovo, requerimentos nutricionais

Introdução

A intensificação do melhoramento genético tem promovido ganhos consideráveis no desempenho produtivo de codornas japonesas nos últimos anos (Reda et al., 2015). De maneira geral, as aves têm apresentado crescimento mais rápido, se tornaram mais pesadas, mais produtivas e com ovos maiores (Rostagno et al., 2017). No entanto, devido à essas mudanças, torna-se necessário a constante atualização das exigências nutricionais (Hasanvand et al., 2018), uma vez que a nutrição correta é essencial para permitir que as aves expressem todo o seu potencial genético de produção.

Sabe-se que a produção de ovos é dispendiosa em termos de nutrientes, visto que todos, incluindo os aminoácidos, devem estar presentes em quantidades suficientes no ovo para o desenvolvimento do embrião (Everaert et al., 2007). Nesse momento, a principal fonte de nutrientes para a produção de ovos é a dieta, uma vez que as reservas corporais não são suficientes para atender a demanda. Sendo a proteína bruta (PB) considerada o principal nutriente da ração, quando deficiente, pode haver limitação da síntese dos componentes do ovo (NRC, 1994). Nesse caso, as aves podem compensar reduzindo o tamanho ou o número de ovos postos, consequências que podem se prolongar por vários dias, principalmente se a limitação ocorrer na fase inicial da produção (Agboola et al., 2016). Por outro lado, os prejuízos provocados pelo fornecimento de dietas com níveis inadequados de PB para codornas em postura não se restringem apenas aos níveis abaixo da exigência. O excesso de consumo desse nutriente também é prejudicial à produção, uma vez que o metabolismo energético passa a ser direcionado para a excreção do excesso de nitrogênio (Karaalp et al., 2009).

Na literatura podem ser encontradas recomendações para PB na dieta que vão de 20% a 26% para as fases de cria e recria (Murakami et al., 1993; Omidiwura et al., 2016) e de 16% a 24% para a fase de produção (Siyadati et al., 2011; Alagawany et al., 2014). Portanto, as exigências ainda não estão bem estabelecidas, sendo necessários mais estudos para elucidar o melhor nível proteico para o máximo desempenho de codornas japonesas (Ghazaghi et al., 2012). Sendo assim, objetivou-se verificar a influência de diferentes níveis de PB na dieta sobre o desempenho produtivo e qualidade de ovos de codornas japonesas.

Material e métodos

Local, animais e delineamento experimental

O experimento foi conduzido no Departamento de Zootecnia da Universidade Federal de Lavras - MG, Brasil, entre os meses de março e junho de 2018. Todos os procedimentos experimentais foram aprovados pelo Comitê de Ética em Uso Animal, sob o Protocolo nº 057/2017.

Um total de 360 codornas japonesas fêmeas (*Coturnix coturnix japonica*) de um dia de idade (8,7 ± 0,5 g) foi adquirido de um incubatório comercial. As aves foram alojadas em galpão de alvenaria e igualmente distribuídas em dez gaiolas de criação (50 cm de largura × 70 cm de profundidade × 25,5 cm de altura) onde permaneceram durante a fase de crescimento (1 a 35 dias). As gaiolas eram dotadas de comedouros lineares tipo calha, bebedouros tipo *nipple*, lâmpadas incandescentes 60 W e bandeja coletora de excretas. A temperatura e a umidade do ambiente foram monitoradas com o auxílio de três termohigrômetros (Vec-HTC 1, Vectus Importatum, São Paulo, Brasil) de mínima e máxima distribuídos no galpão, posicionados na altura das aves. A temperatura foi mantida a 38 °C nos três primeiros dias da criação com uso de aquecedor a lenha, reduzindo 0,5 °C por dia até o 28° dia de idade das aves (Muniz et al., 2018). A luminosidade foi de 24h de luz (natural +

artificial) durante os dois primeiros dias de vida, 23h de luz até o 15° e, em seguida, foram mantidas sob 14h de luz e 10h de escuro até o final dessa fase. Durante esse período, as aves receberam dietas experimentais com diferentes níveis de PB (18, 20, 22, 24 e 26%), sendo duas gaiolas com 36 fêmeas cada para cada tratamento.

Aos 36 dias de idade, as aves foram pesadas, homogeneizadas dentro de cada grupo experimental e transferidas para um galpão telado. Nessa fase, as aves foram distribuídas em grupos de nove em gaiolas de postura (32 cm de largura × 38 cm de profundidade × 16 cm de altura) utilizando um delineamento inteiramente casualizado com cinco tratamentos (níveis de PB) e oito repetições, sendo a parcela experimental constituída por nove fêmeas, totalizando 40 unidades experimentais. No início dessa fase, a luz foi aumentada 30 minutos a cada dia até que se atingisse 17h de luz e 7h de escuro, a qual permaneceu até o final do período experimental (Faitarone et al., 2005; Barreto et al., 2007), que foi de 85 dias.

Durante a fase de produção, as aves receberam as mesmas dietas experimentais da fase anterior, porém com teores de PB reduzidos em quatro unidades percentuais (14, 16, 18, 20 e 22%), seguindo as recomendações das Tabelas de Exigências (NRC, 1994; Rostagno et al., 2011). Todas as rações foram isoenergéticas e isonutritivas para os demais nutrientes (Tabelas 1 e 2), formuladas à base de milho e farelo de soja de acordo com as recomendações de Rostagno et al. (2011), exceto para PB, que foram reduzidos ou aumentados em duas unidades percentuais a partir do nível recomendado para ambas as fases (22% para a fase de crescimento e 18% para a fase de produção). Amostras de rações foram retiradas em ambas as fases para a avaliação do teor de PB final. As rações foram fornecidas à vontade durante todo o período experimental.

Características produtivas e reprodutivas das aves

A produção de ovos foi avaliada quando o lote atingiu 50 e 95% de postura. O abate das aves ocorreu no início da fase de produção (36 dias) e no dia seguinte ao registro dos 50 e 95% de postura (48 e 58 dias de idade, respectivamente). Em cada abate, uma fêmea de cada unidade experimental foi selecionada de acordo com o peso médio da parcela, dessensibilizadas pela técnica de deslocamento cervical e abatidas por meio da sangria. As aves foram dissecadas e tiveram o fígado, ovário e oviduto removidos e pesados. Na presença de ovos em formação em qualquer segmento do oviduto, o mesmo foi retirado antes da pesagem. Em seguida, os folículos amarelos foram contabilizados, removidos e o maior folículo foi pesado separadamente, assim como o estroma (ovário sem os folículos amarelos). O oviduto foi esticado em uma superfície plana e teve seu comprimento mensurado com auxílio de uma régua.

Qualidade e composição interna dos ovos

As análises de qualidade interna, externa e composição interna dos ovos foram realizadas em três períodos com intervalo de 14 dias entre os mesmos, sendo que a primeira coleta foi realizada quando o lote atingiu 95% de postura. Inicialmente todos os ovos íntegros coletados no dia foram pesados e posteriormente imersos em água, para o cálculo do peso médio dos ovos e do peso específico, seguindo a metodologia descrita por Freitas et al. (2004). Em seguida, três ovos eram selecionados de acordo com o peso médio da parcela, identificados e pesados individualmente em balança digital com precisão de 0,01g. Os ovos foram quebrados em superfície plana de vidro onde foram mensurados a altura e espessura de gema e altura de albúmen com auxílio de paquímetro digital (Digimess, São Paulo, Brasil). A gema e o albúmen foram separados, pesados, acondicionados individualmente em tubos plástico e armazenados em freezer -20 °C para posteriores análises de matéria seca e proteína.

As cascas foram lavadas e após secagem por 72h foram pesadas em balança digital com precisão de 0,0001 e mensurados as espessuras com auxílio do paquímetro digital (Digimess, São Paulo, Brasil). O peso do albúmen foi obtido por diferença entre o peso dos ovos e os pesos da gema e da casca. A unidade Haugh foi calculada a partir da formula $\hat{H} = H + (7{,}57 - 1.7\ W^{0,37})$, em que, H é a altura do albúmen em milímetros e W o peso do ovo em gramas (Eisen et al., 1962).

As amostras de gema e albúmen foram encaminhadas ao Laboratório de Pesquisa Animal do Departamento de Zootecnia da UFLA, onde foram descongeladas, homogeneizadas e encaminhadas para a determinação dos teores de proteína bruta e matéria seca, seguindo as técnicas descritas por Silva and Queiroz (2002).

Análise estatística

Os dados foram submetidos ao teste de normalidade (Anderson-Darling), homocedasticidade (Breusch-Pagan) e independência dos erros (Durbin-Watson). Ao satisfazer as premissas de normalidade, análise de variância (ANOVA) foi realizada e, quando significativa ao nível de 5%, análise de regressão para os níveis de PB. Em casos de falta de ajuste na análise de regressão linear (R^2<0,70), análise de Broken Line foi realizada para se determinar o melhor nível de proteína (Wen et al., 2016). Quando as premissas da ANAVA não foram atendidas e as transformações de dados de Box-Cox e de Johnson não foram efetivas na tentativa de normalizar os dados, os mesmos foram submetidos à análise não paramétrica e as médias comparadas pelo teste de Kruskal-Wallis. Toda análise estatística foi realizada utilizando os programas estatísticos Statistica versão 13.3 e Action versão 3.5.

Resultados

Características produtivas e reprodutivas

O aumento da PB da dieta aumentou de maneira linear (P<0,01) o peso vivo das aves em todas as idades avaliadas (Tabela 3). Maior peso do fígado foi observado (P<0,01) aos 36 dias de idade quando dietas com 23% de PB foram utilizadas na fase de cria. Já aos 58 dias, o aumento da PB da dieta reduziu (P<0,01) de maneira linear o peso relativo desse órgão até o nível de 22% de PB na fase de cria e 18% na fase de produção (Figura 1). Não houve efeito (P>0,05) da PB da dieta sobre o peso do fígado aos 48 dias de idade.

Dietas com 24 e 26% de PB na fase de cria resultaram (P<0,01) em maior peso do maior folículo e maiores pesos relativos do estroma, ovário e oviduto das aves aos 36 dias de idade. Aos 48 dias de idade, a PB da dieta aumentou (P<0,05), de maneira linear, apenas o peso relativo do estroma. Já aos 58 dias de idade, as características anatômicas do trato reprodutivo não foram influenciadas (P>0,05) pelos teores de PB da dieta aos 48 e 58 dias de idade.

A PB da dieta aumentou (P<0,01), de maneira linear, a intensidade de postura das aves aos 48 dias de idade. Entretanto, nenhum efeito (P>0,05) foi observado nessa variável aos 58 dias de idade.

Qualidade dos ovos

Os níveis de PB da dieta aumentaram (P>0,05) de maneira linear o peso dos ovos aos 51, 71 e aos 85 dias de idade (Tabela 4). Efeito linear também foi observado na densidade do ovo aos 57 dias e na altura da gema aos 71 e 85 dias de idade. A porcentagem de casca reduziu (P<0,05) de maneira linear aos 71 e 85 dias de idade. Não houve diferenças (P>0,05) na porcentagem de gema, porcentagem e espessura de albúmen e unidade Haugh em nenhuma das idades avaliadas.

Tanto a matéria seca quanto a proteína da gema e do albúmen, não foram influenciados (P>0,05) pelos níveis de PB da dieta (Tabela 5).

Discussão

O aumento do teor de PB da dieta mostrou-se eficiente em aumentar a produção de ovos das codornas apenas até os 48 dias de idade (50% de postura), entretanto, efeitos positivos no peso dos ovos, sem afetar a qualidade interna, foram observados até o final do período mensurado.

Vários estudos têm demonstrado que a produção de ovos está diretamente relacionada ao teor de PB da dieta, tanto em galinhas (Mohiti-Asli et al., 2012; Ghasemi et al., 2014) quanto em codornas (Freitas et al., 2005; Li et al., 2011). Sabe-se que a PB dietética representa a principal fonte de aminoácidos tanto para a mantença quanto para o crescimento e produção. A proteína dietética digerida libera na corrente sanguínea aminoácidos livres que são utilizados diretamente para a produção de proteína endógena ou como fontes de nitrogênio para a síntese de outros aminoácidos necessários para a síntese proteica. A eficiência com que a síntese proteica ocorre no organismo está diretamente relacionada ao *pool* de aminoácidos no sangue (Scanes et al., 1977; Scanes, 2014). Para codornas em crescimento, assim como para outras aves, o aporte de aminoácidos é primeiramente utilizado para atender as exigências de mantença antes que seja destinado às necessidades de crescimento (Wei et al., 2011). Dessa forma, as maiores taxas de síntese de proteína muscular ocorrem somente quando a PB da dieta atende as exigências tanto de mantença quanto de crescimento. No presente estudo, dietas com menores níveis proteicos resultaram em codornas mais leves no início da fase de produção. Provavelmente, a deficiência aminoacídica teve efeito limitante no desenvolvimento corporal das aves durante a fase de crescimento. Para evitar que a deficiência exclusiva de um aminoácido pudesse influenciar o desenvolvimento

das aves, a relação entre os principais aminoácidos (lisina, metionina, treonina e triptofano) foi mantida entre as diferentes dietas experimentais.

Sabe-se que o peso corporal das aves está diretamente relacionado ao desenvolvimento do trato reprodutivo das aves e, consequentemente, à idade à puberdade (Renema et al., 1999). Estudos mostram que existe relação entre a velocidade de crescimento e a idade à maturidade sexual (Renema et al., 1999; Retes et al., 2019). Uma vez que o trato reprodutivo completa seu desenvolvimento, os folículos aumentam sua capacidade esteroidogênica. Esse fato explica a influência da PB da dieta sobre o início da idade reprodutiva das aves. Se o desenvolvimento dos pequenos folículos ocorre mais cedo, a maturidade sexual dessas aves pode ser antecipada, aumentando a capacidade produtiva das aves, o que de fato, foi observado no presente estudo em aves com 48 dias de idade. Soares et al. (2003) observaram que codornas alimentadas com 18% de PB durante a fase de crescimento iniciaram a postura cerca de quatro dias depois do que as codornas alimentadas com 20, 22 e 24% de PB. No presente estudo, as aves que receberam dietas com 18, 20 e 22% nessa fase apresentaram desenvolvimento retardado do trato reprodutivo, sugerido pelo reduzido peso do estroma, folículo e oviduto aos 36 dias de idade.

O maior nível de folículos ovarianos nas aves que receberam dietas com maiores níveis de PB (24 e 26%) até os 36 dias de idade pode estar relacionado ao maior peso relativo do fígado nessa idade. Sabe-se que desenvolvimento folicular aumenta as concentrações de 17 β-estradiol no plasma e isso estimularia o aumento síntese de componentes da gema no fígado (Renema et al., 1999). Aves em produção, de fato, apresentam normalmente um aumento do tamanho do fígado devido à produção e acúmulo de triglicerídeos utilizados na produção da gema (Yannakopoulos et al., 1995). Por outro lado, o aumento do peso relativo do fígado nas aves que receberam os menores níveis de PB na dieta pode ter ocorrido em consequência da redução no peso vivo das aves, que provavelmente alterou a proporção músculo:vísceras.

Nessas aves, o aporte de aminoácidos pode ter sido insuficiente para atender as exigências de crescimento muscular.

Diversos estudos têm demonstrado também que o aumento dos níveis de PB da dieta está relacionado ao aumentado o peso e porcentagem de gema (Djouvinov and Mihailov, 2005; Reis et al., 2011; Alagawany et al., 2014; Agboola et al., 2016). No presente estudo, embora a porcentagem de gema não tenha sido influenciada em nenhuma das idades avaliadas, o peso dos ovos aumentou de maneira linear com o aumento dos níveis de PB da dieta. Esse resultado pode estar relacionado ao maior peso das codornas. Ozdemir and İnci (2012) também observaram que codornas mais pesadas apresentaram maior peso dos ovos. O aumento nos níveis de proteína, portanto, influencia o crescimento das codornas com consequências diretas no desenvolvimento do trato reprodutivo e subsequente produção de ovos. Além disso, a proteína da dieta, quando deficiente, limita a síntese dos componentes do ovo. Nesse caso, as aves podem compensar reduzindo o tamanho dos ovos ou aumentando o intervalo de postura (Agboola et al., 2016). No presente estudo, os níveis de PB da dieta influenciaram a produção de ovos apenas no período anterior ao pico de postura. Após o pico, apenas o tamanho dos ovos foi influenciado pelos níveis desse nutriente na dieta. Outros estudos também têm demonstrado que o aumento dos níveis de PB da dieta está relacionado ao aumento da produção (Djouvinov and Mihailov, 2005; Reis et al., 2011; Alagawany et al., 2014; Agboola et al., 2016), do peso e da massa de ovos (Agboola et al., 2016; Ratriyanto et al., 2017). Silva (2003) sugere ainda que a deficiência de PB da dieta além de reduzir a produção, pode até comprometer a longevidade produtiva da ave, o que leva ao descarte precoce do plantel.

Apesar de a manipulação dietética ter influência sobre produção de ovos, a proporção dos componentes internos praticamente não é alterada. Isso se deve, principalmente, à forma de transporte de nutrientes do fígado para a gema, através da VLDLy (*very low density*

lipoprotein) e vitelogenina, que são muito resistentes às variações (Speake et al., 1998). Sendo assim, dietas com altos níveis de PB podem aumentar o peso do ovo sem prejuízos à sua qualidade nutricional. Por outro lado, o aumento do tamanho do ovo normalmente não é acompanhado pelo aumento da quantidade de casca (Novak et al., 2006; Reis et al., 2011). Isso explica a menor proporção de casca observada nos ovos provenientes de aves que consumiram dietas com maiores níveis de PB. A formação da casca assim como dos demais componentes do ovo, estão diretamente relacionados ao metabolismo proteico. No entanto, a taxa de deposição desse componente parece ser menos influenciada pelos níveis dietéticos desse nutriente (Novak et al., 2006; Agboola et al., 2016).

A falta de padronização das linhagens de codornas em virtude do constante trabalho de melhoramento genético, além de estudos realizados em diferentes regiões do mundo tem contribuído para as variações dos resultados de desempenho quando se determinam as exigências nutricionais dessas aves (Vali et al., 2005; Omidiwura et al., 2016; Rostagno et al., 2017). A maioria das recomendações atuais (NRC, 1994; Rostagno et al., 2017) propõem 24% de proteína para a fase de crescimento e 20% na fase de produção. No presente estudo, no entanto, os níveis ótimos de PB na dieta que conferiram maior peso dos ovos nas diferentes etapas do crescimento foram de 26% até os 35 dias de idade e de 20% na fase de produção. Além disso, as codornas que receberam esses níveis de PB na dieta iniciaram a postura mais cedo.

Conclusão

O teor de PB na dieta influencia a produção e a qualidade de ovos codornas japonesas. Para aumentar a produção de ovos até o pico de postura, recomenda-se 26% de PB na fase de crescimento e 22% na fase de produção. Para aumentar o peso dos ovos sem comprometer a produção, recomenda-se manter 22% de PB até os 90 dias de idade ou mais.

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399 Tabela 1: Composição centesimal e níveis nutricionais calculados das rações para codornas
400 japonesas nas fases de crescimento (1 a 35 dias).

	Proteína bruta (%)				
	18	20	22	24	26
Milho	56,30	52,40	48,55	44,70	40,80
Farelo de soja	28,80	33,89	38,95	44,03	49,10
Farelo de trigo	2,00	2,00	2,00	2,00	2,00
Fosfato bicálcico	1,240	1,192	1,145	1,098	1,050
Calcário calcítico	1,35	1,35	1,35	1,35	1,35
Óleo vegetal	3,50	3,58	3,65	3,73	3,80
Sal comum	0,399	0,399	0,398	0,397	0,397
Suplemento vitamínico[1]	0,100	0,100	0,100	0,100	0,100
Suplemento mineral[2]	0,100	0,100	0,100	0,100	0,100
DL-Metionina (99%)	0,005	0,038	0,070	0,103	0,136
L-Lisina (78%)	0,052	0,039	0,026	0,013	0,000
L-Treonina (99%)	0,002	0,015	0,031	0,046	0,060
L-Triptofano (98%)	0,004	0,003	0,002	0,001	0,000
Cloreto de colina (60%)	0,050	0,050	0,050	0,050	0,050
Caulim	6,00	4,75	3,50	2,25	1,00
TOTAL	100,00	100,00	100,00	100,00	100,00
Composição nutricional calculada					
Energia metabolizável (kcal/kg)	2900	2900	2900	2900	2900
Proteína bruta (%)	18,00	20,00	22,00	24,00	26,00
Proteína bruta analisada (%)	18,32	20,09	21,92	24,14	26,39
Lisina digestível (%)	0,881	0,998	1,115	1,232	1,349
Metionina+Cistina digestível (%)	0,500	0,566	0,630	0,696	0,761
Treonina digestível (%)	0,613	0,692	0,773	0,854	0,934
Triptofano digestível (%)	0,202	0,229	0,256	0,282	0,309
Cálcio (%)	0,900	0,900	0,900	0,900	0,900
Fósforo disponível (%)	0,333	0,333	0,333	0,333	0,333
Sódio (%)	0,176	0,176	0,176	0,176	0,176
Relação aminoácidos/Lisina					
Lisina (%)	100	100	100	100	100
Metionina+Cistina (%)	56	56	56	56	56
Treonina (%)	69	69	69	69	69
Triptofano (%)	23	23	23	23	23

401 [1]Enriquecimento por kg de ração: 10 mg de cobre (mín); 50 mg de ferro (mín); 1,2 mg de iodo (mín); 80 mg de
402 manganês (mín); 0,28 mg de selênio; 60 mg de zinco (mín);
403 [2]Enriquecimento por kg de ração: 0,8 mg de ácido fólico; 35 mg de ácido pantotênico; 10 mg de biotina; 40 mg
404 de niacina; 11,500 UI de vitamina A; 3,0 mg de vitamina B1; 22 UI de vitamina E; 0,6 mg de vitamina B12; 4,4
405 mg de vitamina B2; 10,0 mg de vitamina B6; 2100 UI de vitamina D3; 1,5 mg de vitamina K3; 125 mg de
406 antioxidante.

Tabela 2: Composição centesimal e níveis nutricionais calculados das rações para codornas japonesas na fase de produção (36 a 90 dias)

Ingredientes	Proteína bruta (%)				
	14	16	18	20	22
Milho	61,10	57,05	53,00	48,95	44,90
Farelo de soja	19,35	24,39	29,43	34,47	39,50
Farelo de trigo	3,00	3,00	3,00	3,00	3,00
Fosfato bicálcico	1,153	1,107	1,06	1,014	0,967
Calcário calcítico	6,784	6,785	6,785	6,788	6,790
Óleo vegetal	2,600	2,675	2,75	2,825	2,900
Sal comum	0,323	0,323	0,322	0,321	0,320
Suplemento vitamínico[1]	0,100	0,100	0,100	0,100	0,100
Suplemento mineral[2]	0,100	0,100	0,100	0,100	0,100
DL-Metionina (99%)	0,427	0,516	0,604	0,692	0,780
L-Lisina (78%)	0,373	0,366	0,358	0,349	0,340
L-Treonina (99%)	0,037	0,038	0,038	0,038	0,038
L-Triptofano (98%)	0,023	0,020	0,016	0,013	0,009
Cloreto de colina (60%)	0,037	0,037	0,037	0,037	0,037
Caulim	4,50	3,45	2,30	1,20	1,00
TOTAL	100,00	100,00	100,00	100,00	100,00
Composição nutricional calculada					
Energia metabolizável (kcal/kg)	2800	2800	2800	2800	2800
Proteína bruta (%)	14,71	16,71	18,71	20,71	22,71
Proteína bruta analisada (%)	14,07	16,20	18,04	20,01	21,99
Lisina digestível (%)	0,809	0,927	1,058	1,162	1,279
Metionina+Cistina digestível (%)	0,664	0,761	0,868	0,953	1,049
Treonina digestível (%)	0,485	0,556	0,638	0,697	0,767
Triptofano digestível (%)	0,170	0,195	0,220	0,244	0,269
Cálcio (%)	0,909	0,909	2,909	0,909	0,909
Fósforo disponível (%)	0,303	0,303	0,303	0,303	0,303
Sódio (%)	0,176	0,176	0,145	0,176	0,176
Relação aminoácidos/Lisina					
Lisina (%)	100	100	100	100	100
Metionina+Cistina (%)	82	82	82	82	82
Treonina (%)	60	60	60	60	60
Triptofano (%)	21	21	21	21	21

[1]Enriquecimento por kg de ração: 10 mg de cobre (mín); 50 mg de ferro (mín); 1,2 mg de iodo (mín); 80 mg de manganês (mín); 0,28 mg de selênio; 60 mg de zinco (mín);

[2]Enriquecimento por kg de ração: 7300 UI de vitamina A; 2120 UI de vitamina D3; 8500 UI de vitamina E; 2 mg de vitamina K3; 1,1 mg de vitamina B1; 3,5 mg de vitamina B2; 2,1 mg de vitamina B6; 5,05 µg de vitamina B12; 25,5 mg de niacina; 10 mg de ácido pantotênico; 100 µg de biotina; 18 mg de antioxidante.

Tabela 3. Características reprodutivas e desempenho produtivo de codornas japonesas fêmeas recebendo dietas com diferentes níveis de proteína bruta nas fases de crescimento (de 1 a 35 dias de idade - 18, 20, 22, 24 ou 26%) e produção (de 1 a 90 dias de idade - 14, 16, 18, 20 ou 22%).

Variável	Proteína bruta (%)					SEM	P =
	18/14	20/16	22/18	24/20	26/22		
	- 36 dias de idade -						
Peso das aves (g)	88,4*	104,4	110,3	119,6	120,9	2,10	<0,01
Peso do fígado (%)	2,77**	2,49	2,16	2,23	2,41	0,12	0,01
Número de folículos	0,00 b	0,00 b	0,00 b	1,13 ab	1,75 a	-	0,01
Peso do maior folículo (%)	0,00 b	0,00 b	0,00 b	0,09 a	0,12 a	-	0,01
Peso do estroma (%)	0,00 b	0,00 b	0,00 b	0,10 a	0,13 a	-	0,01
Peso do ovário (%)	0,07 b	0,08 b	0,07 b	0,31 a	0,38 a	-	<0,01
Peso do oviduto (%)	0,03 b	0,06 b	0,09 b	0,73 a	1,05 a	-	0,01
	- 48 dias (50% de postura) -						
Peso das aves (g)	143,8*	158,2	160,5	160,7	163,5	1,98	<0,01
Peso do fígado (%)	3,69	3,36	3,22	2,95	2,94	0,24	0,17
Número de folículos	4,88	6,75	5,38	5,88	5,25	0,60	0,24
Peso do maior folículo (g)	1,81	2,14	2,53	2,55	2,51	0,15	0,31
Peso do estroma (%)	0,24*	0,25	0,35	0,33	0,40	0,04	0,02
Peso do ovário (%)	3,38	3,25	4,09	3,95	4,21	0,44	0,43
Peso do oviduto (%)	1,66	1,49	1,48	1,55	1,47	0,16	0,88
Comprimento do oviduto (mm)	26,45	27,85	30,64	31,69	30,23	1,53	0,12
Produção de ovos (%)	33,93*	51,34	56,70	87,50	87,50	6,74	<0,01
	- 58 dias (95% de postura) -						
Peso das aves (g)	141,7*	157,8	153,9	157,5	164,2	2,84	<0,01
Peso do fígado (%)	3,62*	3,50	3,05	2,81	2,63	0,13	<0,01
Número de folículos	4,86	4,29	4,75	4,25	4,38	0,23	0,34
Peso do maior folículo (g)	2,05	1,85	2,33	2,26	2,44	0,10	0,14
Peso do estroma (%)	0,35	0,39	0,37	0,43	0,45	0,04	0,46
Peso do ovário (%)	3,45	2,55	3,37	3,05	3,14	0,30	0,24
Peso do oviduto (%)	3,47	3,96	4,26	3,68	4,05	0,32	0,45
Comprimento do oviduto (mm)	30,58	31,13	33,41	33,29	33,86	1,42	0,37
Produção de ovos (%)	94,64	91,07	98,51	96,43	94,64	3,92	0,75

* Efeito linear (P<0,05)

** Efeito quadrático (P<0,05)

[a,b] Médias seguidas por diferentes letras na linha diferem pelo teste de Kruskal-Wallis (P<0,05)

Tabela 4. Qualidade interna e externa de ovos de codornas japonesas fêmeas que receberam dietas com diferentes níveis de proteína bruta nas fases de crescimento (de 1 a 35 dias de idade - 18, 20, 22, 24 ou 26%) e produção (de 1 a 90 dias de idade - 14, 16, 18, 20 ou 22%).

Variável	Proteína bruta (%)					SEM	P =
	18/14	20/16	22/18	24/20	26/22		
			- 57 dias -				
Peso do ovo (g)	9,65*	10,49	10,66	10,67	10,98	0,13	<0,01
Densidade do ovo	48,25*	64,50	63,25	63,75	72,13	2,36	<0,01
Gema (%)	27,97	28,47	27,17	27,51	28,22	0,48	0,33
Casca (%)	8,19	8,17	8,23	8,17	8,06	0,15	0,94
Albúmen (%)	63,84	63,36	64,59	64,31	63,72	0,49	0,42
Espessura da casca (mm)	0,18	0,17	0,18	0,18	0,19	0,01	0,26
Altura do albúmen (mm)	4,98	4,84	5,00	4,81	5,12	0,12	0,35
Altura da gema (mm)	11,05	10,96	11,09	10,92	11,26	0,14	0,45
Unidade Haugh	93,20	92,00	92,48	91,95	93,07	0,56	0,38
			- 71 dias -				
Peso do ovo (g)	10,68*	11,59	11,73	11,85	12,07	0,12	<0,01
Densidade do ovo	104,8	123,3	109,3	108,0	127,3	4,53	0,09
Gema (%)	28,87	28,27	29,22	30,27	28,92	0,50	0,40
Casca (%)	8,39*	8,18	8,04	7,57	7,65	0,12	0,02
Albúmen (%)	62,74	64,16	62,60	61,69	63,42	0,51	0,21
Espessura da casca (mm)	0,18	0,19	0,19	0,18	0,18	0,01	0,83
Altura do albúmen (mm)	5,24	5,25	5,31	5,31	5,38	0,12	0,98
Altura da gema (mm)	11,17*	11,40	11,47	11,54	11,61	0,06	0,02
Unidade Haugh	94,13	93,31	93,62	91,86	91,72	1,07	0,73
			- 85 dias -				
Peso do ovo (g)	10,40*	10,98	11,59	11,79	12,00	0,09	<0,01
Densidade do ovo	113,00	144,25	126,75	164,00	146,25	8,77	0,09
Gema (%)	28,50	29,62	28,65	28,68	29,84	0,40	0,35
Casca (%)	8,29*	8,40	7,92	7,88	7,88	0,08	<0,01
Albúmen (%)	63,21	62,47	62,95	63,43	62,28	0,43	0,64
Espessura da casca (mm)	0,14	0,16	0,16	0,15	0,16	0,01	0,42
Altura do albúmen (mm)	5,12	5,10	5,17	4,68	5,36	0,12	0,13
Altura da gema (mm)	11,05*	11,20	11,62	11,72	11,66	0,11	0,02
Unidade Haugh	93,50	92,39	93,33	90,20	93,57	0,61	0,17

* Efeito linear (P<0,01)

Tabela 5. Composição interna dos ovos de codornas japonesas recebendo dietas com diferentes níveis de proteína bruta nas fases de crescimento (de 1 a 35 dias de idade - 18, 20, 22, 24 ou 26%) e produção (de 1 a 90 dias de idade - 14, 16, 18, 20 ou 22%).

	Proteína bruta (%)					SEM	P =
	18/14	20/16	22/18	24/20	26/22		
		- 57 dias -					
Matéria seca da gema (%)	53,1	53,3	52,8	53,5	52,8	0,10	0,33
Proteína bruta na gema (%)	31,1	30,8	31,1	31,5	31,1	0,13	0,66
Matéria seca do albúmen (%)	13,0	13,1	13,5	13,4	13,1	0,15	0,15
Proteína bruta no albúmen (%)	88,8	87,0	85,7	89,6	87,3	1,32	0,08
		- 71 dias -					
Matéria seca da gema (%)	53,7	53,3	52,9	53,0	52,9	0,13	0,32
Proteína bruta na gema (%)	31,5	31,3	31,6	30,9	31,0	0,20	0,67
Matéria seca do albúmen (%)	12,8	13,4	13,1	13,0	12,9	0,08	0,49
Proteína bruta no albúmen (%)	86,2	85,6	85,6	84,9	84,9	1,64	0,98
		- 85 dias -					
Matéria seca da gema (%)	53,0	52,8	53,2	52,8	53,0	0,08	0,84
Proteína bruta na gema (%)	31,1	31,8	30,6	30,8	30,3	0,18	0,07
Matéria seca do albúmen (%)	12,5	12,9	13,0	12,8	12,8	0,03	0,30
Proteína bruta no albúmen (%)	85,7	85,3	86,8	85,6	84,7	1,13	0,63

Não significativo ao teste F (P>0,05)

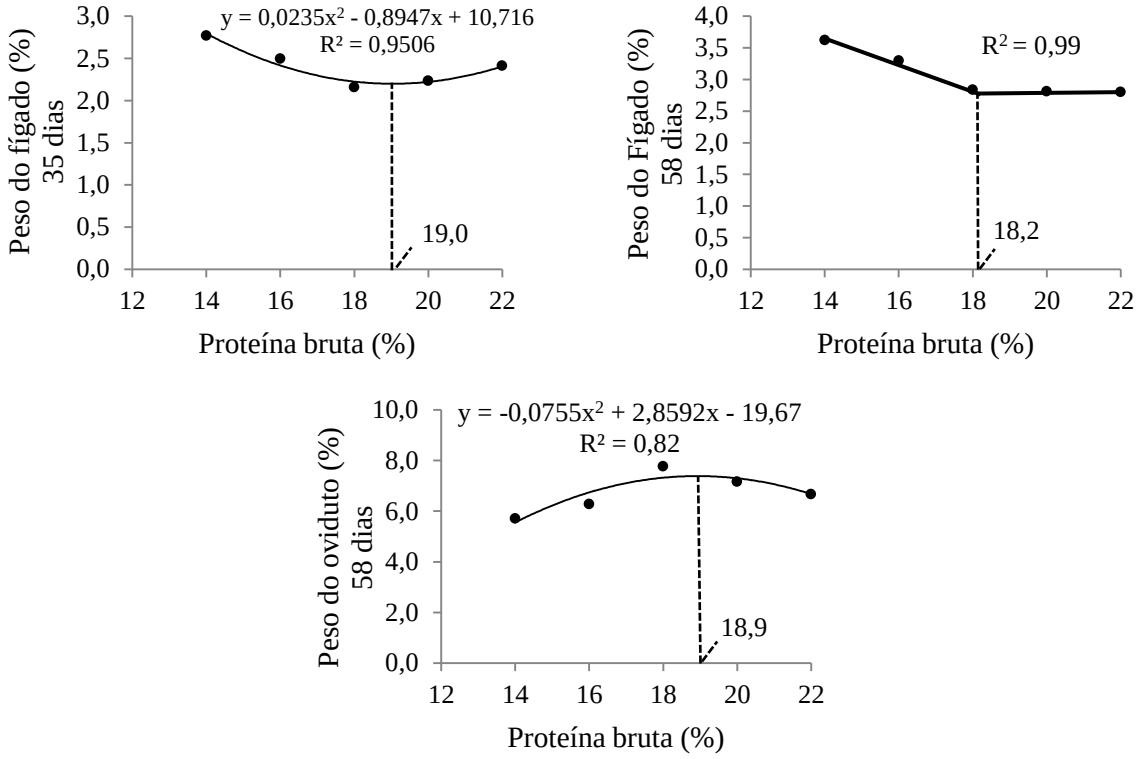

Figura 1. Peso de vísceras de codornas japonesas fêmeas em diferentes idades recebendo dietas com diferentes níveis de proteína bruta nas fases de crescimento (18, 20, 22, 24 ou 26%) e produção (14, 16, 18, 20 ou 22%). O eixo X está apresentando apenas os níveis de proteína bruta utilizados na fase de produção.

ANEXO 1

Atestado de aprovação pela CEUA

UNIVERSIDADE FEDERAL DE LAVRAS
PRÓ-REITORIA DE PESQUISA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS
Cx.P.3037 - Lavras – MG – 37200-000 – (35) 3829-5182 cba@nintec.ufla.br

ATESTADO DE APROVAÇÃO PROVISÓRIO

(o certificado definitivo será concedido após o cumprimento de todos os critérios exigidos pela Orientação Técnica nº 5, de 27 de abril de 2015, do CONCEA/MCTI)

Atestamos que a proposta intitulada "Influência de diferentes níveis de proteína bruta da dieta sobre desempenho reprodutivo de codornas japonesas (*Coturnix coturnix japonica*)", protocolo nº 057/17, sob a responsabilidade de Márcio Gilberto Zangeronimo, Pâmela Lacombe Retes, Renata Ribeiro Alvarenga, Antônio Carlos Cunha Lacreta Junior, Danusa Gebin das Neves e Bárbara Azevedo Pereira, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto homem), para fins de ensino e/ou pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas edificadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), do Ministério da Ciência, Tecnologia e Inovação (MCTI), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da Pró-Reitoria de Pesquisa/UFLA, em reunião de 28/11/2017, podendo ser iniciada a realização da sua parte experimental.

Vigência da autorização: de 01/01/2018 a 31/12/2018
Finalidade: ( ) Ensino (x) Pesquisa Científica
Espécie/linhagem/raça: Ave / *Coturnix coturnix japonica*
Número de animais aprovados: 940
Peso/Idade: 7g / 1 dia
Sexo: macho e fêmea
Origem dos animais: **aguardando documentação**

Lavras, 28 de novembro de 2017.

Prof. Juliano Vogas Peixoto
Presidente da Comissão de Ètica no Uso de Animais CEUA

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