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## The Effect of Crude Protein Level in Diets on Serum Biochemical Indexes and Intestinal Microflora of Yellow Quails

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**Abstract:** A study was carried out to determine the effect of crude protein level in diets on Serum Biochemical Indexes and Gastrointestinal Micro flora of yellow quails. Four diets with different crude protein levels 17.75%, 19.95%, 21.85%, 24.08% were used to feed 192 yellow quails during 50-day-old to 95-day-old in this experiment. The quails were randomly assigned to 4 groups by similar body weight, each of which was subdivided into 4 replicates of 12 quails each. At 95-day-old, two quails per replicates were selected randomly and slaughtered to collect the blood the caecal contents. The blood sample was used to analysis serum biochemical. The caecal contents was detected the *colibacillus*, *lactobacillus* and *bifidobacterium*. The results showed that ALT of diet 1 was significant low ( $p < 0.05$ ); there was no significant of AST in all treatment ( $p > 0.05$ ). Serum TP of treatment 3 is high than treatment 1 ( $p < 0.05$ ). And serum BUN and P was no significant in all treatment ( $p > 0.05$ ). The *colibacillus* and *bifidobacterium* were decreased and *lactobacillus* increased with the increased in dietary protein though there were no significant difference ( $p > 0.05$ ). These results indicated that the different level of dietary protein could not effect on gastrointestinal microflora significantly but significantly effect on serum biochemical indexes of yellow quails.

**Key words:** Yellow quail, serum biochemical, gastrointestinal microflora

### INTRODUCTION

Producing performance is an important indicator of general health, but it is not always right. Because it will damage the liver of birds and usually have a high production to intake excessive amounts of CP in short time. Blood biochemical characteristics could be very important as indicator traits in breeding for the highest productive performance birds (Peterson *et al.*, 1982; Hassan *et al.*, 2006; Hassaan *et al.*, 2008). Yellow quails were wide used in quail breeding in China. But it is not clear which CP level of diets is the optimum level for them. On the other hand, different diets CP level will effect on the nutritional digestion. Then the mounts of nutrition reached the hindgut will be changed. This could impact on intestinal microflora. The present study was conducted to the effect of crude protein level in diets on Serum Biochemical Indexes and hindgut Micro flora of yellow quails.

### MATERIALS AND METHODS

**Animals and dietary treatments:** In this study, one hundred and ninety two eleven-week-old Chinese yellow quails were randomly assigned to four groups by similar body weight, each of which was subdivided into four replicates of 12 quails each. The quails were housed in wire cages, on a schedule with 16 h light and 8 h darkness. The quails received diets with different crude protein levels 17.75%, 19.95%,

21.85%, 24.08% as four diets. The composition and the calculated nutrient content of the experimental Treatments are presented in Table 1. Feed and water were available freely. The experimental diets were designed as:

**Sample collection and analyses:** Feed samples were dried in an oven of 105°C for 6 h for Dry Matter (DM) determination. The CP was determined by the Kjeldahl method, crude fiber and crude fat according to the AOAC (1984). At 95-day-old, two quails per replicates were selected randomly and slaughtered to collect the blood and the caecal contents. Serum were collected after centrifugation (3000r/min for 15 min), decanted and stored at -20°C until biochemical analyses were done. Commercial kits were used for the determination of the following serum constituents according to the procedure outlined by the manufacturer, Total Proteins (TP), Blood Urea Nitrogen (BUN), Phosphorus (P), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) (Shanghai Jiancheng Bio-Product Co., Ltd., China). The fecal sample (1 g) in cecum was placed in an anaerobic glove box within 1 h of collection and homogenized in prepared asepsis physiological saline and diluted from 10<sup>-1</sup> to 10<sup>-8</sup> fold. Portions (100 µL) of each dilution were spread onto the surfaces of plates which contained the following agar media and were incubated anaerobically at 37°C.

Table 1: Composition (air dry basis, %) and nutrient levels (DM basis, %) of trial diets

Items	Treatments 1 (CP 17.75%)	Treatments 2 (CP 19.95%)	Treatments 3 (CP 21.85%)	Treatments 4 (CP 24.08%)
<b>Ingredients</b>				
Corn	64.00	60.00	56.00	51.00
Soybean meal	27.00	30.00	30.00	31.00
Corn gluten meal	1.00	4.00	8.00	11.00
Wheat bran	2.00	0.00	0.00	0.00
Fish meal	1.00	1.00	1.00	2.00
Soybean oil	1.00	1.00	1.00	1.00
Premix 2	4.00	4.00	4.00	4.00
Total	100.00	100.00	100.00	100.00
<b>Nutrient levels</b>				
CP	17.75	19.95	21.85	24.08
ME/(MJ/kg)	12.08	12.15	12.18	12.16
OM	93.70	92.66	93.92	90.56
EE	3.71	3.69	3.77	3.85
CF	5.52	5.80	5.74	5.43
Ash	6.53	6.73	6.77	6.92

Nutrient levels are calculated value based on the measured values of ingredients. Per kilogram mixed additives provides: VA 120000 IU; VD3 40000 IU; VE 500 mg; VB2 70 mg; VB6 60 mg; VB5 400 mg; Folic acid 20 mg; Pantothenic acid 300 mg; Fe 1800 mg; Cu 200 mg; Mn 1400 mg; Zn 1200 mg; Se 5 mg; Ca 20%; P 4%; NaCl 8%; Met 2.5%; Lys 1.5%

Table 2: Measuring method of gastrointestinal microflora of quails

Medium	Culture	Method	Time/h	Temperature/°C
MacConkey medium	<i>E. coli</i>	Aerobic	24	37
MRS agar medium	Lactic acid bacterium	Anaerobic	48	37
BBLmedium	Bifidobacterium	Anaerobic	48	37

Table 3: Serum biochemical indexes of quails in different dietary protein level

Item	Treatments 1 (CP 17.75%)	Treatments 2 (CP 19.95%)	Treatments 3 (CP 21.85%)	Treatments 4 (CP 24.08%)
Serum AST (KU)	9.45±3.66	7.83±1.78	11.43±02.43	6.25±01.92
Serum ALT (KU)	14.77±3.31 <sup>a</sup>	17.66±2.45 <sup>ab</sup>	30.07±07.86 <sup>b</sup>	20.92±04.90 <sup>ab</sup>
Serum TP (mg/ml)	55.18±4.39 <sup>a</sup>	81.88±7.90 <sup>ab</sup>	95.92±12.83 <sup>b</sup>	75.63±10.00 <sup>ab</sup>
Serum BUN (mmol/L)	0.03±0.00	0.06±0.02	0.03±00.01	0.03±00.01
Serum P (mmol/L)	3.39±0.44	2.46±0.52	2.38±00.56	3.23±00.60

<sup>a,b</sup>Mean values within a row with no common superscript differ significantly (p<0.05)

Table 4: Intestinal microflora of quails in different dietary protein level (lgcfu/g)

Item	Treatments 1 (CP 17.75%)	Treatments 2 (CP 19.95%)	Treatments 3 (CP 21.85%)	Treatments 4 (CP 24.08%)
<i>E. coli</i>	8.42±0.11	8.32±0.400	8.28±0.46	8.34±0.430
Lactic acid bacterium	8.48±0.27	8.59±0.630	8.65±0.56	7.91±0.041
Bifidobacterium	8.43±0.11	8.58±0.550	8.80±0.35	8.70±0.400
Bifidobacterium/ <i>E. coli</i>	1.00±0.02	1.03±0.043	1.06±0.03	1.04±0.030

**Statistical analysis:** Data were analyzed by SPSS 10.00 version for Windows. The differences between groups were determined by Variance Analysis (ANOVA). Duncan's multiple range test was performed when differences were significant. Data were expressed as Means±SEM.

## RESULTS

**Serum biochemical indexes of quails in different treatments:** The results (as in Table 3) showed that GPT of serum in Treatments 1 was significant low (p<0.05) than that in Treatments 3. There was no significant of GOT of serum in all Treatments (p>0.05) but it was highest in Treatments 3 and it was very interesting that GPT of serum was highest in Treatments

3. Serum TP of Treatments 3 is high than Treatments 1 (p<0.05). And serum BUN and P was no significant in all Treatments (p>0.05).

**Intestinal microflora of quails in different dietary protein level:** The results (as in Table 4) showed that The colibacillus and bifidobacterium were decreased and lactobacillus increased with the increased in dietary protein though there were no significant difference (p>0.05).

## DISCUSSION

The feeding of different-protein diet results in biologically significant abnormalities in quails (Okumura and Tasaki, 1969). Alterations in measurements such as

BUN and TP are not unexpected. BUN levels are dependent on dietary protein intake (Ozek and Bahtiyarca, 2004); thus, low-protein diets result in reduced BUN concentrations. Thus the BUN in treatment 3 and treatment 4 was lower than that in treatment 2. This maybe, because that feeds intake of quails was lower in treatment 3 and treatment 4. And BUN of all treatments was high unusually (Shen *et al.*, 2008) indicated that the amino acids were not balance in all treatments. Serum AST and ALT activities (liver enzymes) were used to evaluate liver functions, the increase in their activities were related to the degenerations of hepatocytes or liver damage irrespective of its origin (Chen *et al.*, 1999).

Intestinal microflora is a complex microbial system. The relationship between different microorganisms included coordination and competition. Intestinal microflora acts as an important role in host immune system (Backhed *et al.*, 2005). Undigested nutrients can be accumulated in intestine, then the intestinal microorganisms can be caused growing fast, especially some microorganisms caused disease. Thus intestinal microbial flora can be changed. This can affect host health. Bifidobacteria are typical intestinal beneficial microorganisms and *E. coli* was considered as an alert actor which indicate a change of the intestinal microflora structure to unhealthy. So bifidobacterium/*E. coli* values (B/E value) (Wu *et al.*, 2000) can evaluate the status of intestinal microflora. The results showed that with levels of diets protein was elevated B/E values was increasing and then decreasing, though it was not significant.

**Conclusion:** It is concluded that the different diets protein has affect on the Serum biochemical indexes and hindgut microflora of quails. And excess diets protein damaged animal health.

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