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Effect of Soaked and Fermented African Locust Bean Seeds Meal on the Performance, Organs and Carcass Characteristics of Broiler Chickens

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Abstract: This research was conducted to determine the performance, organs weights and carcass characteristics of broiler chickens fed diets containing soaked and fermented African locust bean (*Parkia biglobosa*) seed meal (SFALBSM). Five diets were formulated in which SFLSBM was included in the diets broilers at graded levels of 0, 7.5, 15, 22.5 and 30% designated as T1, T2, T3, T4 and T5 respectively. Two hundred and twenty five (225) broiler chickens (Marshall Strain) were fed these diets in a completely randomized design (CRD). Each treatment was replicated three times with 15 birds per replicate having forty five birds (45) per treatment. The experiment lasted 8weeks (4weeks for starter phase and 4week for finisher phase). At the end of the experiment, carcass analysis was carried out in which three birds were slaughtered from each replication. The results of performance at starter phase showed were significant differences (P<0.05) in the final body weight (734.25-919.89g), total weight gain (679.13–898.31g) and total feed intake (1572.39-1708.56g). The feed conversion ratio (1.87-2.31) were significantly (P<0.05) better for 15% SFALBSM diet compared to others. The results of performance of birds at finisher phase also showed there were significant differences (P < 0.05) in the final body weights (2312.73-2786.14g), total feed intake (4287.73-4373.88g), feed conversion ratio(3.05 -4.55) and feed cost per kilogram gain (₦222.33–316.70). Broilers fed 15% SFALBSM had significantly higher (P<0.05) in weights compared to others (2786.14g). The values for carcass weight and dressing percentage were also significantly (P<0.05) higher for broilers fed 15% SFALBSM diet (1930.24 and 73.98% respectively). There were significant differences (P<0.05) in breast muscle (17.35%-21.97%), drum stick (10.74-11.60%) and thigh muscles (11.63-13.38%). There were significant differences (P<0.05) in the heart (0.49-0.50%), lungs (0.50–0.70%), liver (1.93-2.50%), pancreas (0.20–0.32%) and kidney weights (0.28-0.38%). Feed conversion ratio and feed cost per kilogram gain were better in broiler chickens fed 15% SFALBSM diets (3.26 and 222.33 ₦/kg gain respectively) compared to others. It was therefore concluded that soaked and fermented African locust bean seeds can be included in the diet of broiler chickens up to 15% without any detrimental effect on performance, carcass and organs weights.

Keywords: African Locust Bean Seed, Soaking and Fermentation, Broiler Chickens, Performance and Carcass Characteristics

1. Introduction

Feed is the most important input in a profitable poultry production. It accounts for 70-80% of total the cost of production [1], [2]. At present, the high cost of conventional feedstuffs has brought about the need to have alternative feedstuffs that can replace the expensive ones in order to reduce the cost of livestock production [3], [4]. Nigeria like many developing countries of the world has protein deficiency gap, especially that of high quality animal protein. This low animal protein intake has very serious implications on the health status and well-being of the citizenry [5]. The

shortage of animal protein intake among the ever increasing human population in the third world countries has long been recognized [6]. Poultry production especially broiler chicken is one of the fastest way of achieving adequate animal protein supply for the Nigerian populace due to their short generation interval and rapid growth rate [7]. This is due to their genetic makeup when adequately nourished and managed [8].

Non conventional feedstuffs offer the best alternatives in our environment for reducing feed cost and therefore a reduction in the cost of meat and animal products [9]. The search for alternative sources of protein from legume crops in lieu of expensive ones has been advocated [10]. However, legume seeds contain anti-nutritional factors like enzymes inhibitors, phytate, oxalates, saponin and polyhenolic compounds, which limit their utilization [11]. Fortunately, remarkable improvement in the nutritive value and quality of legume seeds has been achieved by the application of various processing methods through research efforts [12].

The African locust bean tree is a leguminous plant which produces seed grain that is often cheaper and readily available in northern Nigeria. It grows in the savannah region of Nigeria, to the southern edge of zone[13}, [14]. African locust bean seed is rich in protein and is used as a flavor intensifier for soups and stew [12]. The protein content of seeds varies between 25 and 30% and has the potential to be utilized in livestock feeding [15]. This research is aimed at investigating the nutritive value of soaked and fermented African locust bean (*Parkia biglobosa*) and its effects on growth performance, organs and carcass characteristics seeds meal of broiler chickens.

2. Materials and Methods

2.1. Experimental Site

This experiment was conducted at the Teaching and Research Farm of the Department of Animal Science, Faculty of Agriculture and Agricultural Technology, Kano University of Science and Technology, Wudil. Wudil local Government is located in the Sudan savannah region of Nigeria and the farm is located on latitude 11° 37'N and longitude 8° 58'E at an altitude of 403m above sea level [16].

2.2. Sources of African Locust Bean Seeds

African Locust bean seeds were purchased from Dawakin-kudu weekly market. The market is located in the Southern part of Kano state, Nigeria. The predominant variety of African locust bean tree available in Kano State is *Parkia biglobosa*, which is widely distributed in the area.

2.3. Processing of Locust Bean Seeds Soaking and Fermentation

Two hundred and fifty grams (250g) sample of locust bean seeds were cleaned washed and poured into a container containing 2 litres of portable water. It was allowed to soak for 24 hours, excess water was removed and the sample was allowed to ferment for 3 days in an air tight polythene bags. Fermented seeds were removed after 3 days and spread in aluminium metal trays to sun dry for 3 days.

2.4. Proximate Analysis and Determination of Anti-nutritional Factors

The proximate composition of the raw and soaked and fermented ALBSM samples was carried out according the method of [17]**.** Thisanalysis was conducted at Animal Science Biochemistry laboratory of the Faculty of Agriculture, A.B.U. Zaria. (Table 1). The anti-nutritional factors determination in the raw and processed African locust bean seeds were also were also determined and percent of reduction was also calculated and compared (Table 2).

2.5. Experimental Diets

Five diets were formulated containing soaked and fermented African locust bean seeds meal (ALBSM) at dietary levels of 0, 7.5, 15, 22.5 and 30% designated as Treatment 1, 2, 3, 4 and 5 respectively. Broiler starter and finisher diets were formulated for the experiment (Table 3 and 4).

2.6. Experimental Animals and Design

Two hundred and twenty five (225) day old broiler chicks (Marshall strain) were used for the experiment. Having taking the initial weights, diets were allocated to chicks in a completely randomized design (CRD). Forty five (45) chicks were fed per treatment and each treatment was replicated 3 times with 15 birds per replicate. The birds were raised on deep litter house partitioned into pens measuring 2.10m in length, 2.10 m wide and 1.25m in height. The birds were vaccinated against Newcastle diseases at two (2) and four (4) weeks respectively. Feeds and water were offered *ad libitum.*

2.7. Data Collection

The birds were weighed at the beginning of the experiments and reweighed on weekly basis to determine the weight gain at starter and finishers phases. Consequently, a known quantity of feed was offered daily and the feed consumed by birds was determined by subtracting a leftover from the quantity of feed offered to chickens every day. Birds were placed into an empty bucket and weighed with 10kg weighing scale on weekly basis to determine the daily weight gain. The feed conversion ratios were calculated as ratio of daily feed consumed to daily weight gains. At the end final body weights of birds were also taken and the initial body weight was subtracted for determination of total weight gain of birds. Mortality was recorded as it occurred.

At the end of the experiment, carcass analysis was carried out in which three birds from each replicate were randomly chosen and starved for 24 hours before slaughtering. The final live weights of birds were recorded before slaughtering. The birds were bled by slaughtering, immersed in hot water, defeathered and eviscerated. Carcass weights and meat cut

parts (back, drumstick, thigh and wings) were weighed. All the values obtained were expressed as percentage of the live weight of birds. Similarly, the gut weights (crop, proventriculus, gizzard, small intestine weight, and large intestine weights) were measured using a portable electric digital weighing scale. The weights of visceral organs were also expressed as percentage of body weights.

2.8. Data Analysis

Data generated were subjected to Analysis of variance (ANOVA) using the general linear model of statistical analysis system [18].

Statistical model used was $Y_{ij} = \mu + Pi + eij$, Where:

Y_{ij} = the j^{th} observation of the i^{th} processing of seeds by soaking and fermentation.

μ = the overall estimate of the population mean

P_i = the effect of the i^{th} processed seeds by inclusion in diets (T1, T2, T3, T4 and T5)

E_{ij} = the random error.

3. Results and Discussion

The results of proximate composition of raw and processed African locust bean seeds meal are presented in (Table 1). The protein content was slightly improved (28.56%) and the level of fibre content was also reduced (10.27% compared) to raw seeds (24.31 and 13.55 respectively. [19] Reported that fermentation improved palatability besides upgrading the nutrient composition of the fermented material. The results on anti-nutritional factors with percentage reduction were presented in Table 2. There were some reductions of the level of anti-nutrients when the by soaked and fermented for three days seeds compared to raw. This agreed with work of [20] who reported that soaking and fermentation reduced the level of toxic compound in legume seeds. This could be due to the positive effect of fermentation which led to the improvement of the protein which is presumed to be used by fermenting microbes [21].

The results on performance of broiler chickens during the starter phase (0-4 weeks) are presented in Table 5. The results showed there were significant differences ($P<0.05$) in the final weight again and total weight gain of broilers fed soaked and fermented diets. The final weight gain was better for chicks fed control diets and 15% having 919.89g and 937.81g respectively. While, the lowest value of final body weight was recorded for birds fed 30% SFALBSM diet (734.25g). This is in line with report of [22] who stated that soaking and fermentation facilitates microbial and enzymes action on certain anti-nutritional factor in grain legumes thereby enhancing better utilization of nutrients. The chicks fed T1 and T3 had significantly ($P<0.05$) higher total weight gain compared to others. This finding is supported by [23] who reported that fermentation action products allow for increased utilization of feed by chickens.

The results on performance of broiler chickens at finisher phase fed SFALBSM diets are presented in Table 6. There were significant ($P<0.05$) differences in final body weight and total weight gain of birds. Final body weight of broilers was highest for diet 3 (2786.14g) and the values were similar to those on diets T2 (2743.41g) and T4 (2686.14g). However, diet 5 gave the lowest value (2312.73g) of weight compared to others. This may be attributed to effective utilization of nutrients in the diets. Soaking and fermentation proved to have reduced effectively the level of toxic compounds in the legume seeds [20]. The feed cost per kilogram. gain was significantly ($P<0.05$) better for birds fed Treatment 3 (₦222.33) compared to others. There were significant differences ($P<0.05$) in the feed conversion ratio and better value was recorded at 15% soaked and fermented diets. This may be attributed to feed consumption and utilization of feed by birds [24]. This is also in line with work of [25] who reported that fermented parkia pulps can be effectively used to replace up to 20% of costly maize in the diets of Broiler Chickens with a concomitant reduction in feed costs associated with raising the birds

The results of carcass characteristics of broilers fed SFALBSM diets are presented in Table 7. Carcass weights and dressing percentages were significantly ($P<0.05$) affected the dietary inclusion of SFALBSM in the broiler chickens. Carcass weights were higher for birds fed 7.5 and 15% dietary diets (1942.05 and 1930.24g respectively) compare to broiler chickens fed diet 5 containing 30% SFALBSM (1640.44g). Dressing percentages of birds were also higher in diets 1, 2 and 3 (73.92, 73.10 and 73.98% respectively) compared to others. The higher values of dressing percentages were attributed to feed consumption and utilization of feed by birds [26]. There were significant ($P<0.05$) effects of diets on the breast muscle, drum stick, and thigh muscles. The value on breast muscles was recorded highest in birds fed T3 (21.97%) and lowest for those fed T5 (17.35%). Drum stick was recorded highest in birds fed T2 (12.50%) and lowest for birds fed T3 (10.74%). This result agreed with the work of [27] and that of [28] who reported that processing improves the availability and good utilization of proteins and energy content of legumes seeds. Thigh muscle was significantly ($P<0.05$) highest for chickens fed T3 (13.38%) compared to others. The wings, neck, and abdominal fat weights were not significantly ($P>0.05$) affected by dietary treatments of SFALBSM. The results for organs and guts measurements of broilers fed diets containing graded levels SFALBSM are presented in Tables8. There were significant differences ($P<0.05$) in the heart, lungs, liver, pancreas and kidney weights. This agrees with the findings of [28] and [29] who reported that liver is the organ involved in detoxification. Most of gut measurements were not significantly ($P>0.05$) influenced by the treatments, except the large intestine weight which had recorded higher value for birds fed T5 (0.94%) and this was similar with values observed in control diet and 15% SFALBSM diet (0.91 and 0.92%).

4. Conclusion

Based on this study, It is concluded that soaked and fermented African locust bean seed can be included in the diets of broiler finisher chickens up to 15% dietary level without any depression on growth with significant reduction in the feed cost of production

Table 1. *Proximate compositions of Raw and Soaked and fermented African locust bean seed meal.*

Parameter	Raw (%)	SKFALSM (%)
Dry matter	96.36	93.78
Crude Protein	24.31	28.56
Crude fibre	13.55	10.27
Ether extract	4.97	3.24
Ash	4.85	9.53
Nitrogen Free Extract	52.75	48.69

*SFALBSM= Soaked and fermented African Locust bean Seed meal

Table 2. *Levels of anti-nutritional factors and Percentage reduction by Soaking and fermentation of African Locust bean Seeds (SFALBS).*

Anti-nutritional factors	Raw seeds	Soaked and FermentedSeeds	Percent Reductionof ANFs in SFALBS (%)
Tannin (mg/100g)	1.08	0.11	89.89
Phytate (mg/100g)	0.71	0.17	76.05
Saponin (mg/100g)	2.08	0.45	78.36
Oxalate (mg/100g)	1.78	0.43	75.84
Typsin inhiitor (mg/100g)	0.90	0.15	83.33

Table 3. *Composition of experimental diets for broiler starter containing graded levels of Soaked and fermented ALBSM.*

	%levels of inclusion of SFALBSM				
	T1	T2	T3	T4	T5
Ingredients (%)	(0%)	(7.5%)	(15%)	(22.5%)	(30%)
Maize	53.00	48.50	45.00	42.60	39.00
Groundnut cake	25.00	22.00	18.00	13.50	9.00
SFALBSM	0.00	7.50	15.00	22.50	30.00
Soya bean Full-fat	10.00	10.00	10.00	10.00	10.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Wheat offal	5.00	5.00	5.00	5.00	5.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Salt	0.30	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.25	0.25	0.25	0.25	0.25
Vitamin-mineral premix	0.25	0.25	0.25	0.25	0.25
	100	100	100	100	100
Calculated analysis (%)					
Crude protein	23.00	23.00	23.00	23.00	23.00
M. E Kcal/kg	2944	2945	2954	2987	2980
Crude fibre	3.34	3.59	3.82	4.04	4.25
Ether extract	5.85	5.74	5.60	5.48	5.30
Ash	3.36	3.85	4.30	4.73	5.15
Ca	1.36	1.37	1.38	1.39	1.40
P	0.95	0.95	0.93	0.92	0.91
Lysine	1.21	1.26	1.30	1.34	1.37
Methionine+ cystine	0.58	0.62	0.65	0.69	0.72
Feed cost ₦/Kg	76.30	74.46	74.44	73.68	72.86

*Biomix premix supplied the following per kg of diet: Vit. A,10000 I.U., Vit. D, 2000 i.u; Vit k,2mg Vit.B1(Thiamine), 1.8mg; Vit B2 (Riboflavin), 5.5mg; Vit B6 (Pyridoxine), 0.3mg; Vit B12, 0.015mg; Pantothenic acid, 7.5mg; Folic acid, 0.75mg; Niacin, 27.5mg; Biotin, 0.6mg; Choline chloride, 3000mg; Cobalt, 0.2mg; copper, 3mg; Iodine, 1mg; Iron, 20mg; manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg;

Table 4. *Composition of experimental diets for broiler finisher containing graded levels of Soaked and fermented ALBSM.*

	% Inclusion levels of SFALBSM				
	T1	T2	T3	T4	T5
Ingredients (%)	(0%)	(7.5%)	(15%)	(22.5%)	(30%)
Maize	61.00	57.50	54.00	50.50	46.00
Groundnut cake	18.00	14.00	10.00	6.00	3.00
SFALBSM	0.00	7.50	15.00	22.50	30.00
Soya bean Full-fat	10.00	10.00	10.00	10.00	10.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Wheat offal	4.00	4.00	4.00	4.00	4.00

	% Inclusion levels of SFALBSM				
	T1	T2	T3	T4	T5
Ingredients (%)	**(0%)**	**(7.5%)**	**(15%)**	**(22.5%)**	**(30%)**
Bone meal	3.00	3.00	3.00	3.00	3.00
Salt	0.30	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.25	0.25	0.25	0.25	0.25
Vitamin-mineral premix	0.25	0.25	0.25	0.25	0.25
	100	100	100	100	100
Calculated analysis (%)					
Crude protein	21.00	21.00	21.00	21.00	21.00
M. Energy Kcal/kg	3002	3011	3019	3019	3029
Crude fibre	3.31	3.51	3.44	3.80	4.05
Ether extract	5.71	5.58	5.44	5.30	5.18
Ash	3.06	3.51	3.96	4.41	4.90
Ca	1.35	1.36	1.37	1.39	1.40
P	0.93	0.92	0.91	0.90	0.90
Lysine	1.11	1.15	1.19	1.23	1.29
Methionine+ cystine	0.56	0.60	0.63	0.67	0.71
Feed cost ₦/Kg	74.66	73.67	73.06	72.26	71.06

*Biomix premix supplied the following per kg of diet: Vit A, 10000 I.U; Vit D3 2000 i.u.; Vit E, 23mg; Vit K,2mg; Vit K2mg; B1 (thiamine) 1.8mg; Vit B2 (Riboflavin), 5.5mg; Vit B6 (Pyridoxine),3.0mg; Vit. B12, 0.015mg; Pantothenic acid, 7.5mg; Folic acid, 0.75mg; Biotin, 0.06mg; Choline chloride, 300mg; Cobalt, 0.2mg; copper, 3mg; Iodine, 1mg; Iron 20mg; manganese, 40mg; Selenium 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg; M..E. = Metabolisable Energy and SFALBSM= Soaked and Fermented African Locust Bean Seed Meal.

Table 5. *Performance of broiler chickens fed soaked and fermented African locust bean seed meal diets during starter phase (0-4weeks).*

	Level of Soaked and fermented African locust bean seed meal in Diets						
Parameters	**0%**	**7.5%**	**15.00%**	**22.5%**	**30%**	**SEM**	**P**
Initial body weight (g)	39.50	39.50	39.50	39.50	39.50	0.00	1.01
Final body weight (g)	919.89^{a}	881.87^{b}	937.81^{a}	895.89^{b}	734.25^{c}	19.96	0.02
Total weight gain (g)	880.39^{a}	842.37^{a}	898.31^{a}	723.81^{b}	679.13^{b}	23.98	0.03
Total feed intake (g)	1708.56^{a}	1696.99^{a}	1672.72^{b}	1625.40^{c}	1572.39^{c}	6.24	0.01
Feed conversion ratio	1.94^{b}	2.02^{b}	1.87^{c}	2.23^{a}	2.31^{a}	0.06	0.03
Feed cost ₦/kg gain	148.19^{b}	152.20^{b}	137.51^{c}	163.44^{a}	168.71^{a}	4.72	0.02
Mortality rate (%)	2.22	4.00	4.33	4.44	0.00	3.08	0.82

abc= mean with different superscripts on the same row are significantly different (P<0.05), SEM= Standard error of means, P =probability.

Table 6. *Performance of broiler chickens fed soaked and fermented Africanlocust beanSeed meal diets during finisher phase.*

	Level ofSoaked and fermented African locust bean seed meal in Diets						
Parameters	**0%**	**7.5%**	**15.00%**	**22.5%**	**30%**	**SEM**	**P**
Initial body weight (g)	1350.02	1350.03	1350.02	1350.03	1350.02	0.01	0.73
Final body weight (g)	2627.06^{b}	2743.41^{a}	2786.14^{a}	2686.14^{ab}	2312.73^{c}	35.06	0.01
Total weight gain (g)	1277.04^{b}	1424.73^{a}	1438.76^{a}	1336.16^{ab}	962.72^{c}	32.85	0.02
Total feed intake (g)	4430.35^{a}	4430.91^{a}	4373.88^{a}	4377.33^{a}	4287.73^{b}	26.46	0.02
Feed conversion ratio	3.47^{b}	3.18^{ab}	3.05^{a}	3.26^{ab}	4.55^{c}	0.09	0.03
Feed cost ₦/kg gain	259.31^{b}	235.05^{c}	222.33^{c}	237.63^{bc}	316.70^{a}	6.99	0.04
Mortality rate (%)	2.22	4.00	4.33	4.44	0.00	1.24	0.45

abc= mean with different superscripts on the same row are significantly different (P<0.05), SEM= Standard error of means, P = Probability value

Table 7. *Carcass characteristics of broiler chickens fed soaked and fermented African locust bean seed meal diets at finisher phase.*

	% Inclusion levels ofsoaked and fermented African locust bean seed meal						
Parameters	**T1 (0%)**	**T2 (7.5%)**	**T3 (15.00%)**	**T4 (22.5%)**	**T5 (30%)**	**SEM**	**P**
Final live weight (g/bird)	2621.04^{b}	2740.09^{a}	2780.20^{a}	2680.13^{ab}	2290.11^{c}	30.09	0.01
Carcass weight (g/bird)*	1820.02^{b}	1942.05^{a}	1930.24^{a}	1750.33^{bc}	1640.44^{c}	54.00	0.03
Dressing percentage	73.92^{a}	73.10^{a}	73.98^{a}	70.20^{b}	70.04^{c}	0.36	0.04
Breast muscle	19.55^{b}	19.68^{b}	21.97^{a}	19.87^{b}	17.35^{c}	0.55	0.03
Drum stick	11.52^{a}	12.50^{a}	10.74^{a}	12.29^{b}	11.60^{a}	0.63	0.02
Thigh	11.63^{b}	11.78^{b}	13.38^{a}	12.57^{ab}	11.76^{b}	0.31	0.01
Wings	9.41	9.25	9.94	9.47	9.39	0.46	0.67
Neck weight	6.55	7.89	8.17	7.14	7.9	0.11	0.34
Back weight	21.32^{a}	19.69^{c}	22.23^{a}	19.53^{c}	20.21^{b}	0.76	0.03
Abdominal fat	2.52	2.74	2.62	2.71	2.53	0.12	0.43

abc= mean with different superscripts on the same row are significantly different (P<0.05), SEM= Standard error of means, P = Probability value

Table 8. *Organs and guts weight of broiler chickens fed soaked and fermented African locust bean seed meal diets at finisher phase.*

	% Inclusion levels ofsoaked and fermented African locust bean seed meal						
Parameters	**T1 (0%)**	**T2 (7.5%)**	**T3 (15%)**	**T4 (22.5%)**	**T5 (30%)**	**SEM**	**P**
Heart	0.45^{c}	0.50^{a}	0.50^{a}	0.51^{a}	0.49^{b}	0.01	0.02
Lungs	0.59^{ab}	0.58^{ab}	0.70^{a}	0.50^{b}	0.62^{ab}	0.03	0.04
Liver	2.15^{b}	2.50^{a}	2.26^{ab}	1.93^{c}	1.98^{bc}	0.09	0.03
Pancreas	0.32^{a}	0.20^{b}	0.20^{b}	0.30^{a}	0.28^{a}	0.02	0.01
Kidney	0.28^{b}	0.33^{ab}	029^{b}	0.38^{a}	0.34^{a}	0.02	0.02
Crop	0.09	0.11	0.10	0.09	0.09	0.02	0.38
Proventriculus	0.24	0.20	0.18	0.22	0.21	0.03	0.23
Gizzard (%)	2.76	2.73	2.70	2.72	2.65	0.04	0.30
Small intestine weight	2.20	2.17	2.22	2.22	1.97	0.17	0.78
Small intestine length (cm)	246.01	277.12	272.23	260.41	261.78	9.58	0.44
Large intestine weight	0.91^{ab}	0.83^{b}	0.92^{ab}	0.85^{b}	0.94^{a}	0.05	0.03
Large intestine length (cm)	37.02	35.06	34.53	35.54	34.95	0.78	0.78

abc= mean with different superscripts on the same row are significantly different (P<0.05), SEM= Standard error of mean, P = Probability value.

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