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Probiotic supplementing style effects on the anatomy of Males Akar Putra chicken digestive organs

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Abstract
During the recent years, probiotic has been presented with great growth promoters in chicken production. Thus, the present experiment was carried out to evaluate the anatomical changes of males Akar Putra chicken digestive organs fed on a prepared probiotic (PP) in different supplementing style. The experiment comprised of 10 treatments (24 chicken/treatment), with 3 replicates of each (8 chicken/replicate). The treatments consisted of a control group (T1), PP added to the daily drinking water at the rate 1:1 (1 liter tap water+1g PP) in A1 and 1:2 in A2. PP was added to the diet at the rates 1:1 and 1:2 (1 kg of commercial broiler feed+1, 2 g PP) in T4 and T5, respectively. Furthermore, birds in T6 and T7 were fed on a dry fermented diet with probiotic at the rates 1:1:1 (1 kg of commercial broiler feed+1 liter tap water+1 g PP) and 1:1:2, respectively. Whereas, fermented feed with probiotic in the same previous rates but without drying used to feed the birds in T8 and T9. Birds in T10 were fed on a moist diet at the rate 1:1 (1 liter tap water+1 kg of commercial broiler feed). The results presents that PP treatments had ($p<0.05$) longer esophagus 10-25%, duodenum 25-38%, jejunum 9-70%, ilium 2-64%, cecum 5-31% and colon 7-34% and Total 7-47% than the control group counterparts. In contrast, total GIT weight were 8 to 50% ($p>0.05$) heavier in PP treatments, mainly in the proventriculus and gizzard. In conclusion; the results of current study investigated that using probiotic especially in daily drinking water at rate 1:2 had positive effects on the anatomical observations of most gastrointestinal parts.

Keywords: Akar Putra chicken, probiotic, anatomy, digestive system.

1. Introduction
Akar Putra is a hybrid breed of chicken, created and reared at the University of Putra Malaysia (UPM) by an academic team work. It was as a result of crossing wild jungle fowls and Malaysian village chicken (Ayam kampong) [1].

Probiotics are considered as GRAS (Generally Recognized as Safe) by the Food and Drug Administration organisms (FDA). The main purpose for their use relates to increase the numbers of beneficial microorganisms which will compete the harmful bacteria and reduce their activity [2, 3]. Digestive tract generally consider as a food transporter tube, in chickens consist from mouth, esophagus, crop, glandular stomach (proventriculus), the muscular stomach (gizzard) and intestines. Some of these structures may be vestigial or even lost during the evolution of some species [4]. The gastrointestinal trunk (GIT) segments varied anatomically (length and weight) between the species of birds [5, 6, 7]. These anatomical variation maybe due to differences in the absorbed cells efficiency which extend along the GIT [8]. The morphological traits of the GIT organs were examined first time in turkey [9]. After that many researchers were covered the same traits in many bird species, like pigeon, duck, goose [10] and chicken [11]. Hassouna [10] was revealed that the duodenum length range was (22-35cm) in chicken, (40-49cm) in goose, (22-38cm) in duck, (12-22cm) in pigeon and (29-39cm) in turkey. While, the jejuno-ileum length was 98-138cm in chicken, 170-213cm in goose, 100-158cm in duck, 53-84cm in pigeon and 200-250cm in turkey. The same author stated that the jejunum was the longest part and the ileum was the shortest part of the small intestine in all the experimental birds. Furthermore, the caeca length range in chicken was 12-25cm, in goose was 22-34cm, in duck was 10-20cm and in pigeon was 2-7cm. whereas, in terms of rectum-cloacal length range, it was 8-11cm, 16-22cm, 8-13cm and 3-4cm in chicken, goose, duck and pigeon respectively. Finally, he calculated the total length range of GIT in chicken (152-234cm), goose (279-352cm), duck (150-250cm), pigeon (72-125cm) and turkey (390-500cm). There is no reported document regarding the GIT parts anatomy of Akar Putra Chicken and its

effectiveness by any supplementation. Thus, this study was undertaken with the main aim to define the anatomical structures of Akar Putra chicken GIT parts and its anatomical effectiveness by probiotic in different supplementing style.

2. Materials and methods

2.1 Animals and housing

The chicken farming was occurring at the poultry farm which belongs to the Faculty of Veterinary Medicine in University Putra Malaysia (UPM), Malaysia, for 12 weeks. 240 one-day-old males Akar Putra chicks were isolated by using feathering rate sexing method based on [12]. The birds were assigned randomly to 10 experimental groups (24 chicks per treatment), with 3 replicates of each (8 chicks per replicate). They reared in wire cages with 8 birds per pen (5"x 4"x1.5"). The feed diet as shows in (Table 1) was *ad libitum* offered and the water provided continuously. The birds along the experimental period (12 weeks) were kept under uniform management conditions.

Table 1: Composition of basal diet

Items	Basal Diet	
	1 to 22 d (Start diet)	23 to 84 d (Finisher diet)
Corn	44.9	53.10
Wheat	18.0	15
Soybean meal (45%)	33	27
Mineral and vitamin premix	1	1
Oil	2	3
Limestone	0.8	0.6
Dicalcium phosphate	0.3	0.3
Total	100 %	100 %
Calculated analysis		
Crude protein (%)	21.92	19.70
Metabolism energy (kilo calorie per kg. Diet)	2990	3100
Calcium (%)	0.93	0.85
Phosphorus (%)	0.48	0.45
Methionine (%)	0.55	0.50
Lysine (%)	1.35	1.25
Methionine + Cysteine (%)	0.85	0.91
Folic acid	1.1	1.2

Calculated analysis according to NRC [16].

2.2 Probiotic preparation (PP) and supplementation

Each 1 gram of prepared probiotic (PP) has at least 10^9 CFU (Colony Forming Unit) of *Bacillus subtilis*, *Lactobacillus acidophilus*, *Bifidobacterium* and at least 10^8 CFU of *Saccharomyces cerevisiae*. The fermentation process of the feed was done by mixing commercial broiler feed, probiotic and tap water. These mixtures were kipped in a plastic tray and incubated for 38 hours at 37±2°C. The experimental birds were supplemented prepared probiotic at the following styles:

T1: Control group fed on a diet without probiotic supplementation.

A1: PP dissolved in the daily drinking water at 1:1 rate (1g PP/1liter tap water).

A2: PP dissolved in the daily drinking water at 2:1rate (2g PP/1liter tap water).

B1: PP supplemented in the daily diet at 1:1 rate (1kg of commercial broiler feed+1g PP).

B2: PP supplemented in the daily diet at 1:2 rate (1kg of commercial broiler feed+2g PP).

C1: Birds fed on a dry fermented feed mixture prepared at 1:1:1 rate (1kg of commercial broiler feed+1liter tap water+1g PP)

C2: Birds fed on a dry fermented feed mixture prepared at 1:1:2 rate (1kg of commercial broiler feed+1liter tap water+2g PP)

D1: Birds fed on a wet fermented feed mixture prepared at 1:1:1 rate (1kg of commercial broiler feed+1liter tap water+1gram PP).

D2: Birds fed on a wet fermented feed mixture prepared at 1:1:2 rate (1kg of commercial broiler feed+1liter tap water+2gram PP).

E: Birds fed on a wet feed mixture prepared at 1:1 rate (1kg of commercial broiler feed+1liter tap water).

2.3 Sampling procedure

At week 12, 12 birds per treatment (4 birds/replicate) were selected, slaughtered and the GIT segments from the esophagus to the rectum was carefully excised, identified and analyzed based on the method of Jawad *et al*., [11]. Each part of GIT was cleaned, weighed by an electronic balance (precision = 1 g) and its length was measured with a tape measure (±1 mm). Variation ratio of probiotic treatments than control group was calculated according to the formula reported by Jawad *et al*. [11]:

((A-B)/B)*100

A: treatment data

B: control group data

2.4 Data analysis

SPSS statistical program was used to analyze the obtained results. The experiment followed the complete randomized design and the treatments were compared by one way ANOVA test, at $P < 0.05$ level.

3. Results

Table 2 shows the length variation in males' digestive tract segments between the supplementing treatments and control group. No significant difference has been reported between the treatments and the control group in the length of proventriculus, gizzard and rectum. Whereas, the esophagus was longer ($p<0.05$) in the C2, D1 and D2 treatments. Although, no significant difference was shown with A1, A2, B1, C1 and E treatments. Furthermore, duodenum length was significantly ($p<0.05$) high in A2, A1, B1, C1 and D2 treatments. The effect of supplementing tow grams of prepared probiotic with daily drinking water was prominent through relevant ($p<0.05$) superiority in the length of

jejunum, ilium, cecum, colon and the total GIT length.

Table 3 provides the means and standard error values of GIT parts weights for males in all the treatments. Only proventriculus and gizzard were reported significantly ($p<0.05$) different between the treatments. The superiority order in the proventriculus weight trait was in B1, C1, D1, D2 and E treatments. Even though, they did not significantly differ with A1, A2, B2 and the control group. Furthermore, supplementing tow grams of PP in daily drinking water significantly ($p<0.05$) increased the weight of the gizzard.

Table 2: Mean values of GIT segments length (± S.E) of supplementing probiotic in different styles.

Trt.	Segments length (cm)									
	Eso.	**Pro.**	**Giz.**	**Dud.**	**Juj.**	**Ili.**	**Cec.**	**Col.**	**Rec.**	**Total**
T1	15±0.76^{b}	3.5±0.58	1.9±0.67	21.1±1.21^{bc}	40±2.3f	40.5±2.3^{d}	27.4±1.1^{d}	4.7±0.6^{ab}	3.3±0.6	157.4±10.16^{d}
A1	16.6±0.86^{ab}	3.2±0.37	1.7±0.37	22.9±1.04^{b}	49.8±2.1bcde	43.7±2.1^{c}	33.7±0.9^{abc}	5±0.6^{ab}	3.8±0.4	180.5±8.72^{bcd}
A2	17.4±0.72^{ab}	4.1±0.64	1.9±0.55	27.1±1.27^{a}	68.1±2.4a	66.3±2.6^{a}	36±1.2^{a}	6.3±0.9^{a}	3.6±0.7	230.9±10.77^{a}
B1	16.5±1.18^{ab}	3.1±0.67	2.2±0.76	26.4±1.1^{a}	53.2±2.5bc	53.9±2.3^{b}	32.2±1.3^{bcd}	6.3±0.8^{a}	4.3±0.8	198.2±11.42^{bc}
B2	14.9±1.13^{b}	3.7±0.79	2.1±0.7	21.8±0.99^{bc}	41.3±2.6f	41.2±2.5^{c}	28.8±1de	5.1±0.7^{ab}	3.7±0.4	162.8±10.65^{d}
C1	18±1.15^{ab}	3.7±0.73	2.1±0.64	26.6±0.86^{a}	58.1±2.4b	58.8±2.1^{b}	30.1±1.2^{cde}	5.1±0.7^{ab}	4.2±0.7	206.6±10.48^{ab}
C2	18.8±0.99^{a}	2.9±0.49	1.7±0.78	21.3±1.42^{bc}	44.8±2.2def	44.1±2.4^{c}	29.9±1.1^{cde}	3.8±0.4^{b}	4.2±0.8	171.6±10.44^{cd}
D1	18.5±0.79^{a}	3±0.61	1.8±0.44	20.7±0.91^{bc}	43.7±2.1ef	42.8±2.1^{c}	29.1±1.2^{de}	4.4±0.5^{ab}	4.1±0.6	168.2±9.26^{cd}
D2	18.7±0.91^{a}	3.5±0.55	2.1±0.64	29.2±1.3^{a}	51.9±2.3bcd	54±2.3^{b}	35.2±1.3^{ab}	5.2±0.8^{ab}	4.8±0.9	204.6±10.92^{ab}
E	15.9±0.7^{ab}	3.8±0.42	1.9±0.5	19±1.18^{c}	40.9±2.2f	38.9±2.2^{c}	27.3±1.4^{d}	3.8±0.4^{b}	2.7±0.4	154.2±9.29^{d}

- Trt= treatment; Eso= esophagus; Pro= proventriculus; Giz= gizzard; Dud= duodenum; Juj= jejunum; Ili= ilium; Cec= cecum; Col= colon; Rec= rectum.
- Means within a column with different letters differ significantly ($P<0.05$).

Table 3: Mean values of GIT segments weight (± S.E) of supplementing probiotic in different styles. Means within a column with different letters differ significantly ($P<0.05$).

Trt.	Segments length (g)									
	Eso.	**Pro.**	**Giz.**	**Dud.**	**Juj.**	**Ili.**	**Cec.**	**Col.**	**Rec.**	**Total**
T1	6.4±0.9	4±0.3^{ab}	18.8±0.7^{e}	4.7±0.3	8.3±0.2	5.7±0.1	3.2±1	1.1±0.1	2.2±0.1	54.4±4.1
A1	6.6±0.9	4.1±0.4^{ab}	20.1±0.5^{de}	5.1±0.1	9±0.9	7.8±0.6	4.3±0.4	0.9±0.04	3.1±0.2	61.2±3.1
A2	6.8±0.7	4.2±0.4^{ab}	30.1±0.5^{a}	7.1±0.1	11.2±1.1	11.2±1	5.1±0.1	1.1±0.1	5±0.4	81.9±5.1
B1	6.8±0.5	5±0.3^{a}	25.2±0.4^{b}	6±0.2	10.3±0.9	8.7±0.7	4.2±0.3	1±0.03	2.1±0.1	69.3±6.7
B2	7.6±0.7	4.1±0.3^{ab}	24±0.6^{b}	6.8±0.5	9.2±0.2	8.2±0.6	4.3±0.2	0.9±0.04	3.3±0.3	68.5±5.6
C1	7±1.2	5.2±0.4^{a}	21.3±0.4^{cd}	5±0.3	8.4±0.2	7.9±0.6	4.1±0.3	1.2±0.1	3.2±0.2	63.3±3.3
C2	6±0.5	2.9±0.1^{b}	22.1±0.5^{c}	5.1±0.2	8.1±0.3	7.1±0.8	4.1±0.1	1.2±0.1	2.3±0.1	58.8±4.8
D1	6.1±0.4	5.2±0.4^{a}	20±0.6^{de}	5.4±0.4	9.1±0.8	5.2±0.2	4.4±0.3	1.1±0.1	3.2±0.3	59.5±4.6
D2	7±0.5	4.9±0.3^{a}	20.9±0.7^{cd}	5.5±0.4	8.8±0.2	6.4±2	5.5±05	0.9±0.07	2.1±0.2	61.9±5.9
E	5.8±0.3	5.2±0.4^{a}	18.9±0.6^{e}	4.2±0.1	7.5±0.6	5.3±0.3	3.2±0.2	1.1±1	2.2±0.2	53.5±4.6

- Trt= treatment; Eso= esophagus; Pro= proventriculus; Giz= gizzard; Dud= duodenum; Juj= jejunum; Ili= ilium; Cec= cecum; Col= colon; Rec= rectum.
- Means within a column with different letters differ significantly ($P<0.05$).

4. Discussion

Probiotic supplementation in different style had dependent improvement effect on most of GIT segments. The variation ratio of probiotic treatments than control group in esophagus length was as following: C2= 25%; D2=25%; D1=24%; C1=20%; A2=16%; A1=11%; B1=10%; and E=6%. While, in duodenum was D2=38; A2=29%; C1=26%; B1=25%; A1=9%; B2=3% and C2= 1%, with noticeable regression of treatments E=-10% and D1=-2%. The variation ratio of the treatments than control group in jejunum was A2=70%; C1=45%; B1=33%; D2=30%; A1=24%; C2=12%D1=9%; B2=3% and E=2%. Whereas, in case of ilium was A2=64%; C1=45%; B1=33%; D2=33%; C2=9%; A1=8%; D1=6%; B2=2%, with regression in E=-4%. Similarly, the variation ratio of cecum was A2=31%; D2=28%; A1=23%; B1=17%; C1=10%; C2=9%; D1=6%; B2=5% and E=-1%. Furthermore, in colon was A2=34%; B1=33%; D2=11%; B2=9%; C1=8%; A1=7%; C2=-19%; E=-19% and D1=-6%. Total GIT segments variation ratio compared with control group was A2=47%; C1=31%; D2=30%; A1=15%; C2=9%; D1=7%; B2=3% and E=-2%.

In general, the weight of most digestive system parts was affected by probiotic supplementation but not in the significant level. However, proventriculus weight was increased ($p<0.05$) in the probiotic treatments and their variation ratio were C1=32%; D1=31%; E=30%; B1=25%; D2=24%; A2=5%; A1=3%; and B2=3%. Correspondingly, the variation ratio of gizzard was A2=60%; B1=34%; B2=27%; C2=17%; C1=13%; D2=11%; A1=7%; D1=6% and E=1%.

The present results were quit accordance of Windisch *et al*. [13] recorded that many significant effects in the enzymes of digestion, anatomical structure of GIT parts, and immune organs were indicated in birds fed on growth factors as supplements. These improvements in the digestive organs can be justified by increasing the function of the intestine in: digestion and absorption as a consequence to increase the absorptive area [14]. Similarly, Naji *et al*. [15] determined that the relative length and weight of the broiler small intestine and the cecum, as well, were significantly enhanced in the treatments fed on 25, 50, 75 and 100 percentages of fermented fed with probiotic comparing with control group. Furthermore, the author supported the concept that the improvement percentage in these parameters is relating positively with the probiotic supplementing percentage.

Based on the results of current study, it can be suggested that the probiotic increased the numbers of the useful bacteria in the GIT. Consequently, the concentration of the bacteria secondary production, enzymes, will increase. Furthermore, that will increase the probability of diet metabolism, which

will motivate the development of histoanatomical structures of the gastrointestinal tract segments. Lastly, using probiotic will improve the poultry feed conversion ratio which will reflect positively of the other production parameters. These observations, as a whole, results that probiotic is an economical and useful supplementation and can be widely use in the poultry farms.

5. Conclusion

It can be concluded that supplementing probiotic caused significant enhancement in the anatomical characteristics of Akar Putra chicken digestive system. The study demonstrated that supplementing prepared probiotic with daily drinking water at rate 2:1 (2 gram PP/ 1 litter tap water) has high significant and explicit impact on the exploratory birds. It can be suggested that the probiotic will increase the numbers of the useful bacteria in the GIT. Therefore, the concentration of the bacteria secondary production, enzymes, will increase. That will increase the probability of diet metabolism, which will motivate the development of morphometrical structures of GIT segments.

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