Characteristics of immuno-organ development of native chickens fed rations containing hatchery wastes processed with different level of bentonite

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Abstract. Hatchery waste has advantages in the composition of nutritional content, but it has weaknesses on physic-organooleptic appearances, contaminated with Coliform as well as the risk of easy to deceased. Drying, pelletizing and administering adsorbent minerals were appropriate methods on the way of improving its quality. The previous study found that addition bentonite in the processing hatchery waste improved physics-chemicals performances of the products. To these, an experiment was done to study the effect of hatchery wastes with supplementation of bentonite to the immuno-organ development of the animal with the aim to obtain an ideal portion of hatchery waste (HWP) in the rations and its effect to chickens performances. The experiment was conducted by randomized factorial design 2 x 4 x 3 i.e. the level of binder 3 and 4%, the level of hatchery waste on feed formulas 0, 10, 20 and 30%, with 3 replications of each. The result shows an effect of level of bentonite and HWP partially as well as its interaction to the relative weight of thymus and lymph were not significant (p>0.05), but their effect became significant when it was combined with the age of chicken (p<0.05). The level of bentonite and HWP in partial was not significant (p>0.05), but the interaction of bentonite and HWP also interaction both of bentonite and HWP with the age of chicken provided significant effects to the relative weight of bursa fabricius (p<0.05). The relative weight of thymus, spleen, and bursa fabricius tend to decrease with the presence of bentonite in all age determined. However, the initial weight of thymus, spleen, as well as bursa fabricius of chickens remained in normal size. It was concluded that bentonite considered being the binder minerals that improved the safety of chicken hatcheries waste as a local feedstuff. The product was recommended to be applied to the chicken’s diet ages over two weeks.

1. Introduction
Hatchery by-products from hatcheries weights approximately 23 kg per 1000 eggs set at 55-60% moisture. Hatchery waste has good nutrient content. Our previous study noted the hatchery wastes have 60% moisture, 20% crude protein and 9% crude lipid. Calcium content 20-35% and phosphorus content is approximately 0.50%. Several amino acids are available such as methionine, cystine, lysine, iso-leucine. It was reported the protein from the hatchery waste have high biological value, a good balance of amino acids, as well as competitiveness, compared to the prices of soy and fish meal [1,2]. The composition were crude protein (33,1%), crude fat (29%), fibre (12,1%), metabolisable energy (23,9 MJ/Kg), calcium (25,62%) and phosphorus (1,47%). The nutrient composition contributes the microorganism growth so that the hatchery waste may be easily damaged, rotten and unpleasant smell when it does not handle well. Disposal of eggshell hatchery wastes normally not profitable and cost
burden. Therefore, the cost of disposal waste was the undesirable cost [3]. Hatchery waste could be developed into protein sources of feedstuffs, other value-added products or employed as an organic fertilizer after appropriate treatment.

Supplementations of bentonites in the hatchery waste based pellet could improve its proximate component concentration and nutrient availability. The concentrations of ash, crude protein, ether extract and crude fiber of pellet product were increased by increasing level of mineral adsorbent [4]. Moreover, nutrient availability as was represented by apparent metabolizable energy, true metabolizable energy, metabolizability, and nitrogen-retentions were increased by bentonites additions. Some studies showed that the use of sodium bentonite in broiler chickens diet would improve their weight gain. Chickens fed diets containing 1 and 2% sodium bentonite was indicated consume more feed, had more weight gain and less feed conversion ratio [5]. Sodium bentonite as a toxin binder decreases the adverse effect of aflatoxin and causes the improvement of chickens performance [6,7,8,9]. In recent experiments, which assessed the effect of bentonite on blood biochemical parameters, levels of bentonite up to 3% were used in the diet [10,11,12]. However, there was lack on information of bentonite supplementation as well as hatchery waste substitution in the native chickens performances.

It is well understood that non-conventional nutrients can be harmful if not utilized in moderation. Therefore, the following study was conducted to further investigate the effect of dietary supplementation of HWP on growth performance, immune-organ developments in native chickens to gain a better understanding of the application of dietary bentonite supplementation on the way developing of hatchery waste pellet as new feed resources.

2. Materials and methods

2.1. Materials
Hatchery waste that consisting of eggshells (30%), unhatched eggs (60%) and culled neonated chickens (10%) obtained from the commercial hatcheries. Cassava cake meals, bentonite, and other feedstuffs and native chickens are obtained from local suppliers.

2.2. Methods
Experiment was conducted by a factorials completely randomized design 2×4×2 with 3 replications and each replication consisted of 3 experimental units. Hatchery waste treatment refers to the method of Sulistiyanto et al. [13]. The hatchery waste that consist of egg shells (30%), unhatched eggs (60%) and culled DOC (10%) were crushed and blended, then it was added 10% (W/W) cassava cake meals and mixed evenly. After that, the bentonite was added according to the treatment, which is 3% (HWP-B1) and 4% (HWP-B2) and the mixed evenly. The mixture steamed at 80⁰C for 5 minutes and then it was pelletized and dried to simplify the storage. The pellets of hatchery waste (HWP-B1 and HWP-B2) were ground to be mixed with other the components of the treatment ration (0, 10, 20 and 30%). One hundred and fifty DOC unsexed native chickens with initial body weight of 34 ± 4 g were placed in the battery cage according to the treatment (2×4×2×3 @ 3 DOCs). At the age of 0, 2 and 4 weeks, after weighing, the chicken was decapitated. The weight of thymus, lymph and the bursa Fabricius were balanced to observe its development. Data were analysed using analysis of variance to evaluate the effect of treatments[14].

3. Results and discussions
Table 1 shows the effect of treatment to the chicken body weight, the relative weight of thymus, bursa fabricius dan spleen. The body weight of chickens in partial was affected neither the present of bentonite (3 and 4%) nor the level of HWP-B (p=0.05), but the age of chickens both in partial and interactively with the bentonite and the HWP-B influenced the weight of chickens (p<0.05). Chicken body weight tends to decrease with increasing levels of bentonite as well as HWP-B levels and increases with increasing age of chickens.

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Table 1. Effect of Bentonite levels and level of Hatchery Waste Processed in the rations to the body weight, the relative weight of Thymus, Bursa Fabricius and Spleen of Native Chickens

| HWP Levels | HWP-B1 | Average | HWP-B2 | Average |
|-----------|--------|---------|--------|---------|
| Body weight (g) | | | | |
| Age | 0 | 10 | 20 | 30% | 0 | 10 | 20 | 30% |
| 2 wks | 189 | 166 | 157 | 157 | 167,3 | 188 | 161 | 153 | 155 | 164,3 |
| 4 wks | 379 | 305 | 297 | 292 | 318,3 | 370 | 296 | 294 | 229 | 297,3 |
| Average | 284,0 | 235,5 | 227,0 | 224,5 | 279,0 | 228,5 | 223,5 | 192,0 |
| Significance the effect of treatments* | | | | | | | | | |
| ns | A | B | C | AB | AC | BC | ABC |

Relative weight of Thymus (%)

| Age | 0 | 10 | 20 | 30% | 0 | 10 | 20 | 30% |
| 2 wks | 0.60 | 0.53 | 0.39 | 0.44 | 0.49 | 0.51 | 0.53 | 0.65 | 0.49 | 0.55 |
| 4 wks | 0.66 | 0.68 | 0.59 | 0.45 | 0.59 | 0.50 | 0.41 | 0.46 | 0.42 | 0.45 |
| Average | 0.63 | 0.61 | 0.49 | 0.44 | 0.51 | 0.47 | 0.55 | 0.46 |
| Significance the effect of treatments* | | | | | | | | | |
| ns | A | B | C | AB | AC | BC | ABC |

Relative weight of Bursa Fabricius (%)

| Age | 0 | 10 | 20 | 30% | 0 | 10 | 20 | 30% |
| 2 wks | 0.50 | 0.38 | 0.23 | 0.29 | 0.35 | 0.42 | 0.29 | 0.22 | 0.32 | 0.31 |
| 4 wks | 0.55 | 0.40 | 0.22 | 0.45 | 0.40 | 0.54 | 0.48 | 0.37 | 0.32 | 0.43 |
| Average | 0.53 | 0.39 | 0.22 | 0.37 | 0.48 | 0.39 | 0.29 | 0.32 |
| Significance the effect of treatments** | | | | | | | | | |
| ns | A | B | C | AB | AC | BC | ABC |

Relative weight of Spleen (%)

| Age | 0 | 10 | 20 | 30% | 0 | 10 | 20 | 30% |
| 2 wks | 0.13 | 0.17 | 0.19 | 0.12 | 0.15 | 0.16 | 0.19 | 0.26 | 0.23 | 0.21 |
| 4 wks | 0.28 | 0.27 | 0.39 | 0.27 | 0.30 | 0.32 | 0.35 | 0.34 | 0.30 | 0.33 |
| Average | 0.20 | 0.22 | 0.29 | 0.19 | 0.24 | 0.27 | 0.30 | 0.26 | 0.24 |
| Significance the effect of treatments* | | | | | | | | | |
| ns | A | B | C | AB | AC | BC | ABC |

**Note:**

A= Effect of bentonite levels in the HWP (HWP-B1 =3% and HWP-B2=4%),
B= Effect of HWP-B1 or HWP-B2 levels in the rations (0,10,20, 30 and 40%),
C= Effect of chickens age(2 and 4 weeks),
AB= Interaction effect of A and B,
AC= Interaction effect of A and C,
BC= Interaction effect of B and C,
ABC= Interaction effect of A, B and C,
ns = the effect of treatment was not significant (p>0.05 s= ), the effect treatment was significant (P<0.05)

The effect of HWP, bentonite, and age of chickens to the relative weight of thymus, in partial, had not significant (p>0.05), but interactively, the bentonite together with HWP-B level and the age of chickens were significant (p<0.05). Among the treatments, there was no significant effect of level of bentonite in the hatchery wastes nor the hatchery waste processed with bentonite (HWP-B) levels in the ration. Their effects would be significant when interacted with the age of chickens. The relative weight of the thymus tends to decrease with increasing the level of mineral binders. The result noted
that substitution of hatchery wastes containing 3-4% into the ratio up to 30% considered suppressed development of thymus. Effect treatment to the relative weight of bursa fabricius and spleen were in similar with the thymus. The effect was not significant when it was alone, and become significant to the bursa fabricius and spleen when it was interactively (p<0.05). The relative weight of lymph decreased with increasing the level of mineral binders. The age of chickens ameliorated increasing of the relative weight of bursa fabricius and spleen increased. The result noted that substitution of hatchery wastes containing 3-4% zeolite into the ratio up to 30% considered depressed the development of bursa fabricius and spleen of chickens.

An addition of bentonite to the processing of hatchery wastes provided an unsatisfactory effect to the body weight, particularly in the early age of chickens. Supplementation of mineral binders to control the digestive tract microflora was reported able to improve daily gain and feed efficiency of the broilers aged 1-21 days [15,16]. Low initial body weight compared to the broilers in this experiment was addressed to the genetic quality of native chickens that was lower than that of broilers [17]. The contribution of bentonite effects in this study was in line with previous experiment that was note increases of bentonite reduced daily gain and improved the feed conversion of the early age chickens [18], that effect had changed with increasing the age of chickens. Even though it was lower than broiler, the performance of native chickens fed with HWPB has remained better than native chickens fed commercial feeds. In compared to the Thai indigenous chickens, an initial weight of chickens in this experiment was 162 g vs 90 g (2 weeks) and 308 g vs. 213 g (4 weeks) [17]. The bursa Fabricius, thymus, and spleen are lymphoid organs of chickens that play a role in producing lymphocyte cells. The lymphoid tissue seemed to be well underway at hatching, and develop with age of chickens. Effect of feeding ration containing HWPB to the bursa fabricius, thymus, and spleen was in harmony with Indresh et al. [19]. Compared to the control diets, relative weights of thymus, bursal, and spleen of chickens were not altered by supplementation of varying levels of bentonite. Although the binding minerals tend to reduce the weight of spleen, and bursa fabricius, the addition of the binder could restore some of the adverse effects of mycotoxin to the affected parameters significantly [20]. The relative weight of the Bursa fabricius, thymus, and spleen were higher than the Saudi native chickens, Leghorn, and Lohmann at eight weeks of age, which is 0.37 vs 0.13, 0.11 and 0.14% for the Bursa fabricius, 0.53 vs 0.44, 0.28 and 0.40% for the thymus and 0.26 vs 0.17, 0.21 and 0.20% for the spleen [21]. Those could be considered that chicken remains in the growth phase, which is 1-4 weeks of age, therefore, the optimum body weight has not reached.

4. Conclusion
It was concluded that the bentonite considered being the binder minerals that improved the safety of chicken hatcheries waste as a local feedstuff. The product was recommended to be applied to the chicken’s diet ages over two weeks.

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References
[1] Lilburn M S, Barbour G W, Nemasetoni R, Coy C, Werling M and Yersin A G 1997 Poult. Sci 76(1) 841
[2] Mehdipour M, Shams Shargh M, Dastar B, and Hassani S 2009 Pakistan J. Bio. Sci. 12 1272
[3] Glatz P, Miao Z and Rodda B 2011 Handling and treatment of poultry hatchery waste: a review. Sustainability 3 216
[4] Sulistiyanto B, Utama C S and Sumarsih S 2018 J. Indon. Tropic. Anim. Agric. 43(2) 107
[5] Salari S, Kermanshahi H dan Moghaddam H N 2006 Intl. J. Poult.Sci. 5(1) 31
[6] Fairchild A S, Croom J, Grimes J L and Hagler W M 2008 Int.. J. Poult. Sci. 7(12)1147-51
[7] Shi Y, Xu Z, Sun Y, Wang C and Feng J 2009 *Turkish J. Vet. Anim. Sci.* 33 15
[8] Magnoli A P, Tallone L, Rosa C A R, Dalcero A M, Chiacchiera S M and Torres Sanchez R M 2008 *Appl. Clay Sci.* 40 63
[9] Pasha T N, Farooq M U, Khattak F M, Jabbar M A dan Khan AD 2008 *Turk. J. Vet. Anim. Sci.* 32(4) 245
[10] Eraslan G, Essiz D, Akdogan M, Sahindokuyucu F, Altintas LT and E. Hismiogullari S 2006 *Bull Vet Inst Pulawy* 49 93
[11] Kermanshahi H, Hazegh A R and Afzali N 2009 *J. Anim. and Vet. Adv.* 8 1631
[12] Safaei M, Boldaji F, Dastar B, Hassani S, Mutalib M S A and Rezaei R 2014 *Asian J. Anim. and Vet. Adv.* 9 56
[13] Sulistiyanto B, Utama C S and Sumarsih S 2016 *Proc. Intl. Seminar On Livestock Production and Veterinary Technology (Bali)* p 415
[14] Steel R G D dan Torrie J H 1989 *Prinsip dan prosedur statistika suatu pendekatan biometric* (Jakarta :Penerbit PT Gramedia) pp 168-205
[15] Hu C H, Qian Z C, Song J, Luan Z S, and Zuo A Y 2013 *Poult. Sci.* 92 143
[16] Tang Z G, Chen G Y, Li L F, Wen C, Wang T and Zhou Y M 2015 *J. Anim. Sci.* 93 620
[17] Jaturasitha S, Leangwunta V, Leotaragul A, Phongphaew A, Apichartsrungkoon T, Simasathitkul N, Vearasilp T, Worachai L and ter Meulen U 2002 In: *Deutscher Tropentag 2002: Challenges to organic farming and sustainable land use in the tropics and subtropics. Book of Abstracts.* Edited by A. Deininger (Witzenhausen, Germany) pp. 146
[18] Damiri H, Chaji M, Bojarpour M and Mamuei M 2012 *Pak Vet J* 32(2) 197
[19] Indresh H C, Devegowda G, Ruban S W and Shivakumar M C 2013 6(6) 313
[20] Hedayati M, Manafi M, Yari M and Mousavipour S V 2014 *Int. J. Agric. and Forest.* 4(5) 351
[21] Fathi M, Al-Homidan M I, Abou-Emira O K and Al-Moshawah A 2016 *Int. J. Poult. Sci.* 15 (7) 287