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PREFACE

In this edition, volume 25 No 1, we proudly present articles from animal and veterinary sciences including genetics; animal production and feed technology. The articles published in this edition are:
"Direct and Maternal Genetic Trends for Some Productive and Reproductive Traits in Egyptian Buffaloes"; "Effect of N-acetylcystein on ERK Gene Expression in Ovarian Tissue of Acrylamide-Treated Adult Rats"; "Effect of Mix Culture Bacteria and Fungi in Fermented Peanut Hulls-Based Feed Supplement on Physical Quality and *In vitro* Rumen Fermentation Parameters"; "Effects of Two Different Energy Sources in Total Mixed Diets on the Performances and Blood Metabolites of Lactating Boerka Goats"; and "The Effect of *Morinda citrifolia* and *Arthrospira plattensis* Powder on the Performance and Quality of Broiler Duck Carcasses".

We extent high appreciation to all peer reviewers who make this journal academically high value. Hopefully, these articles would offer any benefit to readers and the end-users of technological innovation, and attract interests from scientists to contribute their papers to the Indonesian Journal of Animal and Veterinary Sciences.

Chief Editor

Bogor, March 2020

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LIST OF CONTENT

Direct and Maternal Genetic Trends for Some Productive and Reproductive Traits in Egyptian Buffaloes

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(received 14-11-2019; revised 14-02-2020; accepted 14-02-2020)

ABSTRAK

Abu El-Naser, IAM. 2020. Kecenderungan gen langsung dan maternal beberapa sifat produksi dan reproduksi Kerbau Mesir. JITV 25(1): 1-10. DOI: http://dx.doi.org/10/14334/jitv.v25i1.2069.

Penelitian ini bertujuan untuk melihat kecenderungan fenotip genetik maternal dan genetik langsung pada sifat-sifat produksi seperti produksi susu pertama (FLMY, kg), masa laktasi pertama (FLP, hari), dan produksi susu harian laktasi pertama (FLMD, kg) serta sifat reproduksi seperti umur pertama beranak (AFC, bulan), masa kosong pertama (FDO, hari) dan jarak beranak (FCI, hari). Data yang digunakan merupakan kumpulan data laktasi pertama selama 25 tahun (1991-2015) dari 1.104 ekor pejantan dan 482 indukan yang dipelihara di peternakan Mahallet Mousa milik Lembaga Penelitian Produksi Ternak. Data dianalisis dengan model ternak untuk menentukan parameter penelitian. Nilai tengah dari FLMY, FLP, FLDM, AFC, FDO dan FCI berturut-turut adalah 1.546,5 kg; 189 hari; 7,9 kg; 37,9 bulan; 120,8 hari dan 428 hari. Nilai heritabilitas langsung (h^2a) untuk sifat yang sama secara berturut-turut adalah 0,25; 0,18; 0,24; 0,45; 0,18 dan 0,19. Nilai heritabilitas maternal untuk sifat yang sama secara berturut-turut adalah 0,12; 0,19; 0,22; 0,25; 0,12 dan 0,12. Hubungan genetik (rg) diantara sifat-sifat yang diteliti bervariasi antara -0,19 hingga 0,38. Ketepatan variasi nilai pemuliaan yang diprediksikan adalah antara 69 hingga 94; 0,37 hingga 94 dan 42 hingga 91% untuk FLMY, FLP, FLDM, AFC, FDO dan FCI pejantan, betina dan indukan secara berturut-turut yang mana menunjukkan bahwa pengayaan genetik dapat diaktualisasikan melalui masing-masing pejantan, betina dan indukan. Kecenderungan genetik maternal dan aditif, lingkungan dan fenotip tidak terlihat secara signifikan pada semua sifat. Hal ini mengindikasikan pentingnya penyusunan rencana untuk meningkatkan mutu genetik dan kondisi lingkungan karena mampu meningkatkan produktifitas dan keuntungan.

Kata Kunci: Kerbau, Kecenderungan Genetik Maternal dan Langsung, Kecenderungan Fenotip

ABSTRACT

Abu El-Naser, IAM. 2020. Direct and maternal genetic trends for some productive and reproductive traits in Egyptian buffaloes. JITV 25(1): 1-10. DOI: http://dx.doi.org/10/14334/jitv.v25i1.2069.

This study was done to determine the direct and maternal genetic and phenotypic trends for productive traits such as first lactation milk yield (FLMY, kg), first lactation period (FLP, d) and first lactation daily milk (FLDM, kg), and reproductive traits such as age at first calving (AFC, mo), First days open (FDO, d) and first calving interval (FCI, d). Data were collected over consecutive 25 years (1991 to 2015) of 1104 first lactation of 135 sires and 482 dams maintained at Mahallet Mousa farms of Animal Production Research Institute. Data were analyzed by Animal model to determine genetic parameters for studied traits. Means of FLMY, FLP, FLDM, AFC, FDO and FCI were 1546.5 kg, 189 days, 7.9 kg, 37.9 months, 120.8 days and 428 days, respectively. The direct heritability (h^2a) for same traits were 0.25, 0.18, 0.24, 0.45, 0.18 and 0.19, respectively. Corresponding maternal heritability (h^2m) for mentioned traits was 0.12, 0.19, 0.22, 0.25, 0.12 and 0.12, respectively. Genetic correlations (r_g) among studied traits were varied between -0.19 to 0.38. Accuracy of predicted breeding value varied between 69 to 94, 0.37 to 94 and 42 to 91% for FLMY, FLP, FLDM, AFC, FDO and FCI of sires, cows and dams, respectively that revealed the genetic improvement could be actualized through each of cows or sires or dams. Additive and maternal genetic, permanent environmental and phenotypic trends were not significant for all studied traits. It indicated that it is important to set up a plan to improve genetic and environmental conditions thus, increasing productivity and realization of high profitability.

Key Words: Buffaloes, Direct and Maternal Genetic Trends, Phenotypic Trends

INTRODUCTION

Milk is an important food considered the Egyptian buffaloes an important animal for milk production and meat in Egypt. The number of buffaloes is nearly about 3.9 million. Where contribution to milk production nearly 45.5% of total milk in Egypt (FAO 2013). Liu et al. (2008) shown that the genetic correlations between productive and reproductive traits were negative. To increase genetic and phenotypic improvement in Egyptian buffaloes for milk traits, constructed selection indices (Fooda et al. 2010). The annual genetic trend in

the first three lactations in Egyptian buffaloes for total milk yield, lactation period, calving interval and days open were negative (Shalaby et al. 2016). The conception of genetic progress trend shall help in future genetic direction to be accepted by defining specific goals to breeding profitable and continuity of dairy herd (Missanjo et al. 2012). Genetic trends for milk yield and calving interval were significant and correspond to 1.57 kg/year and 0.058 days/year, respectively and phenotypic trends were 27.74 kg/year and 0.647 days/year, respectively (Ramos et al. 2006). Maternal effects in the analysis models desirable for selection of productive and reproductive traits to optimize hoped for best response over long run (El-Awady et al. 2016). Therefore, the aims of current work were to determine genetic parameters, direct and maternal genetic and phenotypic trends for FLMY, FLP, FLMD, AFC, FDO and FCI in Egyptian buffaloes.

MATERIALS AND METHODS

Data utilized in the present study were collected over consecutive 25 years (1991 to 2015) of 1104 first lactation of 135 sires and 482 dams maintained at Mahallet Mousa Experimental farms (Main Mahallet Mousa, El-Nataf El-Gaded and El-Nataf El-kadim), Animal Production Research Institute (APRI), Ministry of Agriculture. Traits in this study were divided into productive traits namely first lactation milk yield (FLMY, kg), first lactation period (FLP, d) and first lactation daily milk (FLDM, kg), and productive traits namely: age at first calving (AFC, mo), First day open (FDO, d) and first calving interval (FCI, d). Egyptian buffaloes were living under the same system of feeding and management in the stations. Lactating animals were milked twice daily during the lactation period, and milk yield was recorded daily. The animals were fed on Egyptian clover (*Trifolium alexandrinum*) during December to May with concentrate mixture and rice straw. During June to November, animals were fed on concentrate mixture, rice straw and a limited amount of clover hay or silage. The animal was feed according to their live weight, milk production, and pregnancy status. Water is available for buffaloes at all times of the day. Buffaloes were inseminated during heat after 60 days postpartum, while heifers were inseminated when attained 350 kg of live body weight or 18-24 months of age.

Statistical Analysis

Data were analyzed using MTDFREML program of Boldman et al. (1995) with the multiple models to determine genetic and phenotypic parameters for studied traits. As the following model was:

$$Y = X\beta + Za + Mm + Wpe + e$$

Where:

Y, β, a, m, pe and e = a vector of observations, a vector of fixed effects ((Month and year of calving and farm), a vector of direct additive genetic effect, a vector of maternal genetic effect, a vector of permanent environmental effect and e = a vector of residual effect, respectively. However, X, Z, M and W = are incidence matrices relating records to fixed, direct genetic, maternal genetic and permanent environmental effects, successively.

MTDFREML was used to estimate the best linear unbiased perdition (BLUP) of predicted breeding values for all animals.

Genetic trends were obtained by estimation regression the means of predicted breeding values for traits studied on the year of birth as described by (Sahin et al. 2012). The annual phenotypic trend for traits was estimated with regression of least-square means on calving year. Graphs that indicted genetic and environmental trends were made by Microsoft Office Excel. The regression was estimated via SAS program of computer (SAS 2002)

RESULTS AND DISCUSSION

The present unadjusted means of FLMY, FLP, FLDM, AFC, FDO, and FCI were 1546.5kg, 189d, 7.9kg, 37.9mo, 120.8d and 428d, respectively are given in Table 1. The current mean of FLMY was lower than that observed by Madad et al. (2013) being 2220.03 kg in Khuzestan buffaloes and higher than that observed by Shalaby et al. (2016) being 1057kg in Egyptian buffaloes. The present FLP and FCI were shorter than (310.4d and 586.6d, respectively) found by Thiruvenkadan et al. (2014) in Murrah buffaloes. While the current means of FLP, FDO and FCI were longer than that reflected by Shalaby et al. (2016) in Egyptian buffaloes being 226 d, 224d, and 538d, respectively. The present mean of FLMY was lower than (1619.7kg) reversed by Thiruvenkadan et al. (2014) and contrarily for the present mean of FLDM was higher than (5.38 kg) in Murrah buffaloes. While the current means of FMY, AFC and FCI were lower than that observed by Seno et al. (2010) in Murrah buffaloes.

Coefficients of variation for traits in this study were ranging (22.9% to 71.10%) the immense CV % value for FDO (71.10 %), which showed a huge variation between individual buffalo. The present value for CV % for FMY, AFC and FCI was higher than that noticed by Seno et al. (2010) in Murrah buffaloes.

The direct heritability (h^2_a) for FLMY, FLP, FLDM, AFC, FDO and FCI were moderate 0.25, 0.18, 0.24,

Table 1. Means, standard deviation (SD) and coefficient of variation (CV%) for first lactation milk yield (FLMY), first lactation period (FLP), first lactation daily milk (FLDM), age at first calving (AFC), first days open (FDO) and first calving interval (FCI) in Egyptian buffalo

Trait	Mean	SD	CV (%)
FLMY, kg	1546.5	587.7	38
FLP, d	189	45.4	24
FLDM, kg	7.9	3.2	40.5
AFC, mo	37.9	8.7	22.9
FDO, d	120.8	85.9	71.1
FCI, d	428	103.4	24.2

Table 2. Estimate of variance components and heritability for traits under research work

Estimates	Traits					
	FLMY	FLP	FLDM	AFC	FDO	FCI
σ^2_a	9403.76	886.71	306.38	889.66	416.35	465.45
σ^2_m	4521.33	929.05	280.85	490.41	277.09	293.87
σ^2_{pe}	14295.31	1578.51	433.98	216.78	623.63	612.97
σ^2_e	9429.07	1480.49	255.32	378.77	993.15	1079.62
σ^2_p	37612.69	4872.70	1276.17	1970.75	2309.64	2449.15
σ_{am}	-36.78	-2.06	-0.36	-4.87	-0.58	-2.77
r_{am}	-0.006	-0.002	-0.001	-0.007	-0.002	-0.007
h^2_a	0.25	0.18	0.24	0.45	0.18	0.19
h^2_m	0.12	0.19	0.22	0.25	0.12	0.12
c^2	0.38	0.32	0.34	0.11	0.27	0.25
e^2	0.25	0.30	0.20	0.19	0.43	0.44

σ^2_a= additive genetic variance, σ^2_m= maternal variance σ^2_{pe} = permanent environmental, σ^2_e= residual (temporary environmental variance σ^2_p= phenotypic variance, σ_{am}= direct maternal genetic covariance, h^2_a= direct heritability, h^2_m= maternal heritability, c^2= fraction phenotypic variance to permanent environmental e^2= fraction phenotypic variance due to residual effects.

0.45, 0.18 and 0.19, respectively. While the maternal heritability (h^2_m) was moderate for FLP, FLDM and AFC being 0.19, 0.22, 0.25, respectively, While h^2_m for FLMY, FDO and FCI were slightly low and being 0.12 as illustrated in Table 2. In general, obtained low estimated h^2_a for FLMY, FLP, FDO and FCI were 0.14, 0.17, 0.07 and 0.08, respectively in Egyptian buffaloes by Shalaby et al. (2016), Madad et al. (2013) for FLMY was 0.06 of Khuzestan buffaloes, Seno et al. (2010) for FLMY and AFC were 0.20 and 0.07, respectively in Murrah buffaloes and Catillo et al. (2001) for AFC was 0.26 in Murrah buffaloes. As a matter of fact, the estimated permanent environmental ration ranged from 0.11 to 0.38, close to the finding by El-Awady & Abu El-Naser (2017) for MY, LP, DO and CI in Friesian cows. Estimated genetic correlations (r_g) among all traits varied between -0.19 to 0.38. The r_g among FLMY, FLP, and FLDM were positive and varied from 0.10 to 0.38, and the same thing goes for genetic correlations among AFC, FDO and FCI were positive, ranged from 0.03 to 0.24, as shown in table (3). The current results similar to that obtained by Shalaby et al. (2016) in Egyptian buffaloes for r_g between FLMY andFLP was positive but higher than (0.81) present result, as well r_g between FDO and FCI taken the same trend, was positive and higher than current estimation (0.99). Gupta et al. (2015) in Murrah buffalo found r_g between AFC and all of FLMY, FLP and FCI were positive and being 0.18, 0.11 and 0.19, respectively, while r_g between FLP and FCI was 0.59 and r_g between

Table 3. Estimation correlations among traits understudy in Egyptian buffaloes

Trait1	Correlations					
	Trait2	r_g	r_p	r_e	r_{pe}	r_m
AFC	FLMY	0.13	0.02	0.01	0.17	-0.14
	FLP	0.14	0.14	0.04	0.17	0.05
	FLDM	0.19	-0.16	-0.29	0.10	-0.34
	FCI	0.22	-0.14	-0.13	-0.56	-0.12
	FDO	0.24	-0.03	0.05	-0.05	0.04
FLMY	FLP	0.38	0.01	0.10	0.01	-0.22
	FLDM	0.37	0.01	0.19	0.01	0.01
	FCI	-0.01	-0.02	0.01	-0.03	-0.05
	FDO	-0.19	-0.02	0.08	-0.05	0.14
FLP	FLDM	0.10	-0.02	0.17	0.01	-0.46
	FCI	-0.04	-0.17	-0.01	0.02	-0.50
	FDO	0.11	-0.05	-0.03	0.07	0.36
FLDM	FCI	-0.09	-0.13	-0.57	-0.16	0.47
	FDO	0.23	0.07	0.04	-0.28	0.36
FCI	FDO	0.03	0.19	0.42	0.06	0.47

r_g= genetic correlation, $r_{p=}$ phenotypic correlation, r_e = residual environmental ratio, $r_{pe=}$ permanent environmental ratio and r_m= maternal genetic correlation.

FLMY and each of FLP and FCI were 0.86 and 0.49, respectively.

The phenotypic correlations ranged from -0.17 to 0.19 among all traits (Table 3). The current study exceeded that observed by Shalaby et al. (2016) in Egyptian buffaloes, r_p among FCI, FDO, FLMY and FLP were ranged from 0.166 to 0.931. Maternal correlations among studied traits ranged from -50 to 0.46, while the permanent environmental and residual ratios ranged from -0.56 - 0.17, and -0.57 to 0.17, respectively (Table 3).

The portended breeding values (EBV's) through buffalo sires, buffalo cows and buffalo dams for FMY, FLP, FLDM, FDO and FCI are presented in Table 4. The breeding values for FMY, FLP, FLDM, FDO, and FCI of buffalo sires ranged from -444.73 to 397.84 kg, -27.52 to 70.07 days, -3.78 to 3.74 kg, -10.06 to 13.76 months, -44.54 to 46.69 and -35.77 to 46.40 d, respectively. The corresponding value for buffalo cowsays ranged between -424.02 to 596.65 kg , -88.37 to 120.01 days, -4.93 to 3.41 days, -8.29 to 15.87 months, -38.27 to 58.31 days and -32.76 to 57.03 days, respectively. In addition to breeding values for the aforementioned traits of buffalo dams were between -212.08 and 397.18 kg, -53.39 and 62.13 days, -3.15 and 4.83 kg, -9.73 and 10.90, -49.42, and 79.33 days respectively. The ranges breeding values of buffalo cows were higher than those for sires and dams for FLMY, FLP, FLDM, and AFC but the highest value for FDO and FCI were in dams. Accuracy of portended breeding value variation between 69 to 94, 0.37 to 94 and 42 to 91% for sires, cows and dams, respectively, revealed that genetic improvement could be actualized through each of cows or sires or dams. High accuracy levels of breeding values help breeders to select for traits in their buffaloes and from now on genetic improvement in herds. The current results agreed with El-Awady et al. (2016) and El-Awady & Abu El-Naser (2017). In Italian buffaloes, Catillo et al. (2001) reflected that the accuracy for AFC, CI and MY were 0.49, 0.36, and 0.49, respectively. Additive and maternal genetic and permanent environmental trends are shown in Figure 1, 2, 3, 4, 5 and 6. Generally, it is shown that direct and maternal genetic and permanent environmental trends for study traits fluctuated on years.

It is noticeable that the additive genetic value increased to 113.12 kg in year 1999 and decreased to -32.63 kg in year 2011. While permanent environmental value increased to 57.6 kg in year 2014 and decreased to -79.10 in year 1992. It indicated that genetic programs and management can play an important role in the improvement of dairy buffaloes in Egypt.

Table 4. Portended of breeding values for buffalo sires, buffalo cows and buffalo dams and accuracies%, for studied traits

Traits	Breeding Values			
	Minimum ± SE	Maximum ± SE	Accuracy, %	Range
Buffalo sires (EBV's)				
FLMY	-444.73± 6.97	397.84±7.35	67-71	842.57
FLP	-27.52±1.18	70.07±1.76	74 -89	97.59
FLDM	-3.78±0.60	3.74±0.60	94 -94	7.52
AFC	-10.06 ± 2.28	13.76± 2.58	70-78	23.82
FDO	-44.54±1.70	46.69±1.38	70-81	91.23
FCI	-35.77±1.53	46.40±1.29	69-79	82.17
Buffalo cows (EBV's)				
FLMY	-424.02±6.10	596.65±5.90	79-80	1020.67
FLP	-88.37±1.45	120.01±1.42	83-74	208.38
FLDM	-4.93±1.00	3.41±1.05	74-82	8.34
AFC	-8.29±1.27	15.87±1.58	90-94	23.86
FDO	-38.27±2.20	58.31±2.01	38-53	96.58
FCI	-32.76±1.98	57.03±1.97	37-39	89.79
Buffalo dams (EBV's)				
FLMY	-212.08±8.21	397.18±8.84	45-59	609.26
FLP	-53.39±8.89	62.13±2.32	42-44	115.52
FLDM	-3.15±0.74	4.83±0.74	90-.91	7.98
AFC	-9.73±3.16	10.90±3.16	49-50	20.63
FDO	-49.42±1.66	79.33±1.68	70-71	128.75
FCI	-53.13±1.48	106.01±1.45	72-73	159.14

Table 5. Estimates of annual additive, maternal and environmental permanent trends for studied traits in Egyptian buffaloes

Traits	Additive	Maternal	Permanent
FLMY	-0.749	-0.052	2.24
FLP	0.039	-0.104	0.061
FLDM	-0.063	-0.023	0.033
AFC	-0.104	0.068	-0.035
FDO	-0.100	-0.043	0.058
FCI	-0.143	-0.067	0.006

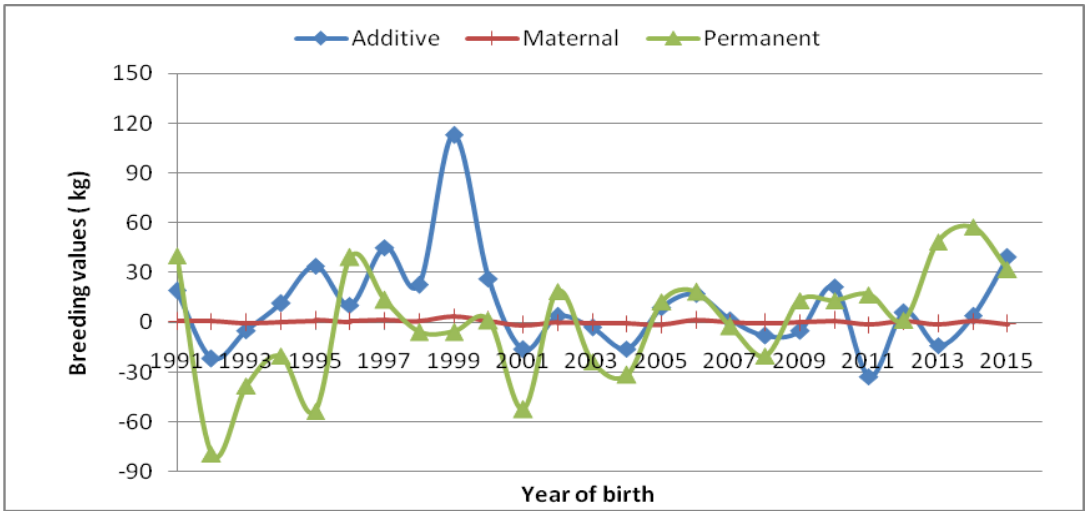

Figure 1. Additive and maternal genetic and permanent environmental for FLMY

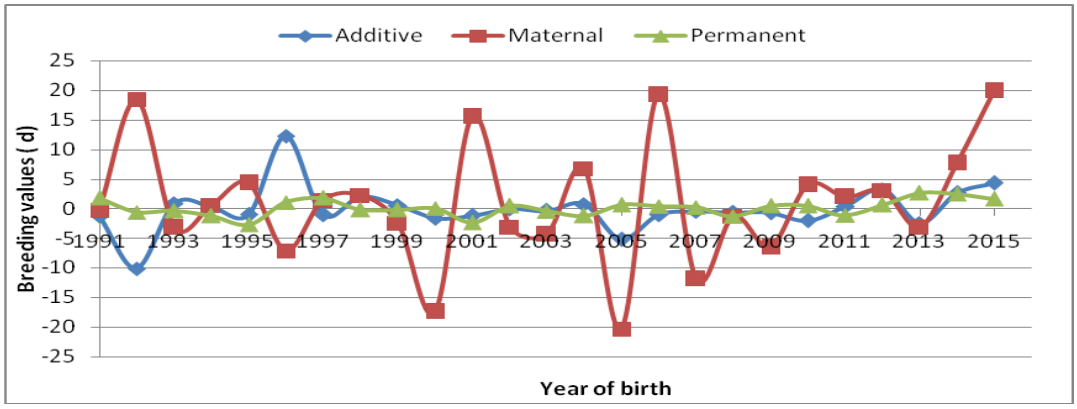

Figure 2. Additive and maternal genetic and permanent environmental for FLP

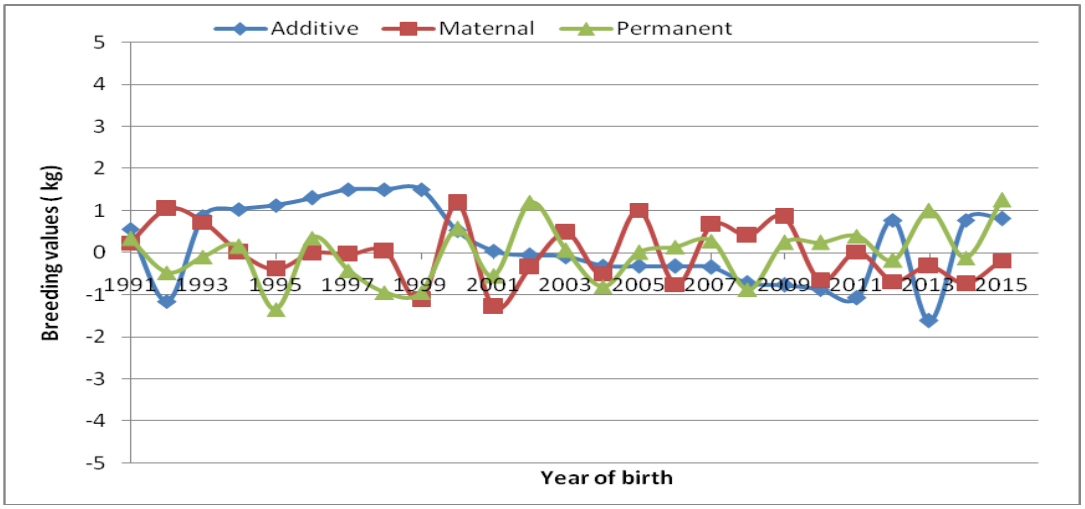

Figure 3. Additive and maternal genetic and permanent environmental for FLMD

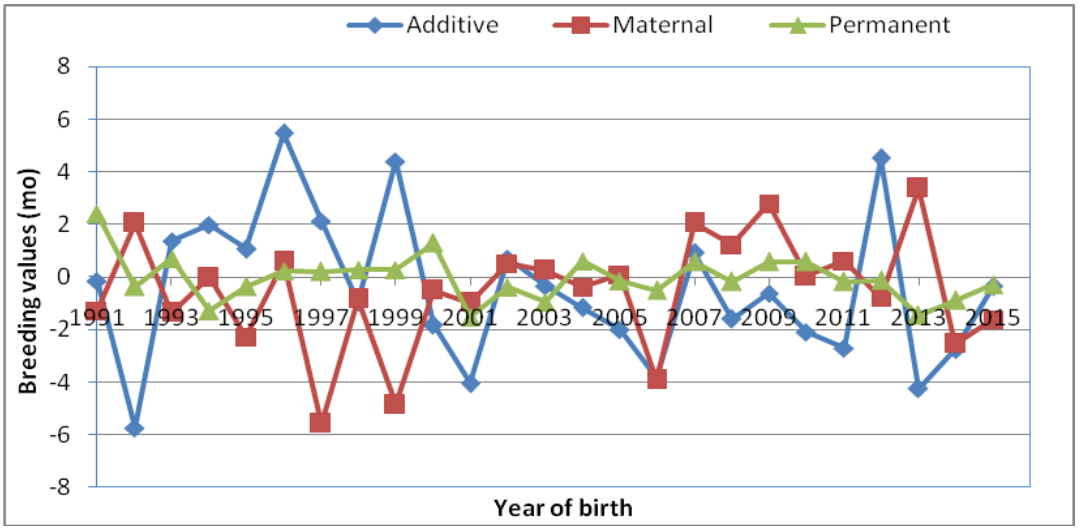

Figure 4. Additive and maternal genetic and permanent environmental for AFC

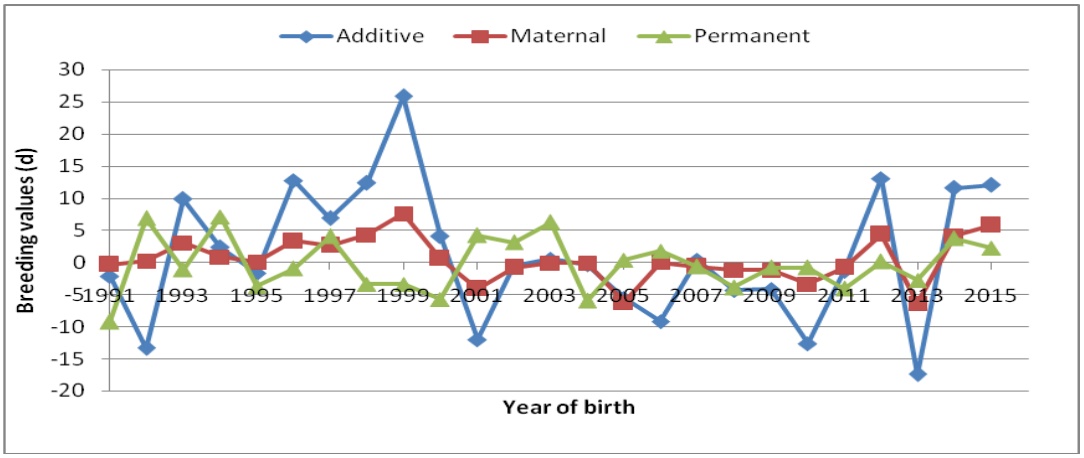

Figure 5. Additive and maternal genetic and permanent environmental for FCI

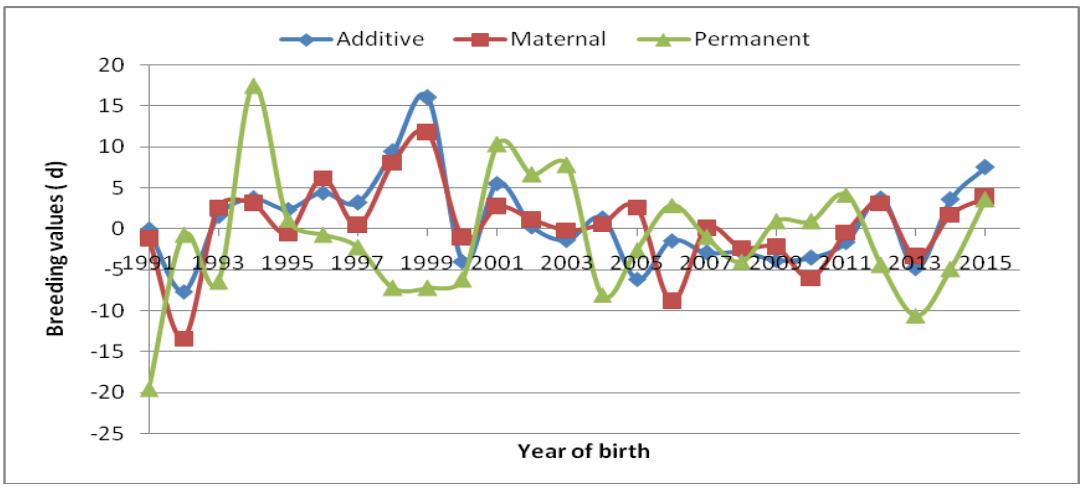

Figure 6. Additive and maternal genetic and permanent environmental for FDO

Table 6. Estimates of annual phenotypic trends for studied traits in Egyptian buffaloes

Year	FLMY, kg	FLP, d	FLDM, kg	AFC, mo	FDO,d	FCI, d
1994	1621	196.8	8.2	45.9	106.2	418.6
1995	1560	193.7	8.1	45.9	41.3	357.7
1996	1686	205.0	8.5	28.5	157.5	496.0
1997	1664	188.8	8.7	33.2	109.0	429.8
1998	1414	169.5	8.6	45.7	124.8	421.8
1999	1436	194.0	7.0	38.6	101.8	417.9
2000	1588	196.1	7.9	42.0	124.6	426.6
2001	1278	159.5	6.9	38.3	96.5	424.2
2002	1614	192.4	8.3	41.4	152.6	449.1
2003	1335	183.0	7.3	37.1	158.8	471.3
2004	1622	189.2	8.4	35.3	118.2	416.5
2005	1394	182.6	7.6	37.1	125.9	437.6
2006	1431	180.8	7.7	38.7	130.8	435.1
2007	1526	186.8	7.9	37.9	100.5	413.7
2008	1687	202.7	8.1	37.5	122.6	429.2
2009	1689	189.4	8.8	37.0	119.2	417.8
2010	1542	185.3	7.9	37.1	97.3	413.4
2011	1455	183.8	7.5	36.3	133.9	436.7
2012	1475	186.9	7.6	36.6	132.6	442.4
2013	1759	198.0	8.6	34.6	93.4	407.1
2014	1766	204.5	8.4	46.4	91.5	395.1
2015	1629	194.3	8.3	37.3	131.1	429.2
b-reg	4.29	0.168	0.0022	- 0.122	0.343	- 0.313

Similarly, noticed by El–Awady et al. (2017) for MY, LP, CI and DO in Friesian cows. The annual additive genetic trends for productive traits (FLMY, FLP, and FLMD) were non-significant and being -0.749 kg, 0.03 day and -0.063 kg. Also annual additive trends even though negative for reproductive traits (AFC, FDO and FCI) were non-significant -0.104 month, -0.100 day and -0.143 day. Corresponding annual maternal genetic trends for productive and productive studied traits were non-significant and being -0.05 kg, -0.104 day, -0.023 kg, 0.068 month, -0.043 day and -0.067 day, respectively. The reasons of this might be consulted to lock from animal selection or increased of culling of many preeminent buffaloes in previous years due to a greater age and ingress of many replacement heifers with less breeding value in the herd or using bulls do not have good breeding values for studied traits in insemination inside the farm. The present results similar to that reflected by Salem & Hammoud (2016) for genetic trends of sires for FMY, FLP and FDO in Holstein were negative and non-significant and he added that may be attributed for selecting sires. The coefficient of permanent environmental trends for above mentioned traits were non-significant and positive expect AFC was negative (2.24 kg/year, 0.061 day/year, 0.033 kg/year, -0.0.35 month/year, 0.058

day/year and 0.006 day/year), respectively. It shows that the permanent environment had no clear effect on studied traits. That may be due to the animals in the farm was kept under control system environmental conditions and management and the betterment of the environmental conditions to desire to simplify selection programs.

Shalaby et al. (2016) obtained that the annual genetic tends for FLMY, FLP, FDO, and FCI were negative -15.80kg, -6.55d, -5.658d and -5.801d, from 1972 to 2002 in Egyptian buffaloes. Fooda et al. (2010) found the annual genetic trends for milk yield in all Mahallet Mousa farms were positive and being 0.58 kg in Egyptian buffaloes. El-Arian et al. (2012) obtained that the annual genetic change for TMY and LP was positive and being 3.70 kg and 0.55 day, respectively as the regression coefficient of sire breeding values per year in Egyptian buffaloes.

Annual phenotypic trends for studied traits in Egyptian buffaloes are illustrated in Table 6. Noticeable fluctuations were monitored for studied traits in different years. Corresponding estimation of annual phenotypic trends for FLMY, FLP, and FLDM were non-significant and positive. This probably refers to improvement partial in environmental conditions. Phenotypic trends for AFC and FCI were no significant and negative directions, pointing out declining trends in AFC and FCI. The alike phenotypic trend for FDO was non-significant, but positive, which is not desirable. Similarly, noticed by El-Arian et al. (2012) for MY, LP and DO in Friesian cows. In Egyptian buffaloes, Fooda et al. (2010) obtained the annual phenotypic trend for milk yield was 26 kg being in all Mahallet Mousa farms. El-Arian et al. (2012) obtained that the annual phenotypic trends for TMY and LP were highly significant and positive, (74.20 kg and 18.84 d), respectively. Over and above, they added that phenotypic improvement in milk yield was carried out during the study and shown the variation in performance between years due to different of management, nutritional and climatic conditions during the period of study in Egyptian buffaloes.

CONCLUSION

The results indicate that the direct heritability estimate for studied traits was moderate. Therefore, the ability to increase the efficiency of those traits through genetic improvement and environmental conditions is needed on the farm at the same time. Additionally, increase and ranges accuracy estimated of breeding values for studied traits inferred being more genetic difference among individuals and increase the possible selection for those traits. Moreover, genetic, phenotypic and Environment values trends fluctuated over the year and non-significant for all studied traits. It is proved that designed genetic programs, management and environmental conditions not satisfactory during the period. It is shown that it is important to set up a plan to the improvement of genetic and environmental conditions in herd Egyptian buffaloes.

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Effect of N-acetylcystein on ERK Gene Expression in Ovarian Tissue of Acrylamide-Treated Adult Rats

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ABSTRAK

Naimi M, Shariati M, Naeimi S, Edalatmanesh MA. 2020. Pengaruh N-acetylcystein terhadap ekspresi gen ERK pada jaringan ovarium tikus dewasa yang mendapat perlakuan akrilamida. JITV 25(1): 11-18. DOI: http://dx.doi.org/10.14334/jitv.v25i1.2161.

Akrilamida (AA) merupakan komponen karsinogenik yang diproduksi selama proses memasak. Penelitian ini bertujuan untuk melihat pengaruh N-asetilsisten (NAC) terhadap ekspresi gen kinase pengatur sinyal ektraseluler (ERK) pada perubahan histopatologi ovarium tikus yang mendapat perlakuan akrilamida (AA). Sebanyak 36 tikus betina Wistar dewasa dikelompokkan secara acak ke dalam 6 kelompok yaitu kontrol, kontrol positif (+VE Con), kontrol negatif (-VE Con), eksperimental 1 (Exp1), eksperimental 2 (Exp2) and eksperimental 3 (Exp3). Dua puluh delapan hari setelah perlakuan, tingkat ekspresi gen ERK diukur menggunakan metode PCR *real-time* dan kemudian dilakukan pemeriksaan terhadap perubahan histopatologi ovariumnya. Tingkat ekspresi gen ERK menurun secara signifikan pada kelompok +VE Con, Exp1 dan Exp2 dibandingkan dengan kontrol ($p<0,05$) tetapi tidak pada kelompok -VE Con dan Exp3 ($p>0,05$). Secara histologi, kelompok +VE Con menunjukkan penurunan jumlah yang signifikan pada folikel primer, sekunder dan Graafian juga *corpus luteum* dibandingkan dengan kelompok kontrol ($p<0,05$) tetapi berbeda pada kelompok negatif, Exp2 dan Exp3 ($p>0,05$). Pada kelompok Exp1, jumlah folikel primer dan sekunder juga *corpus luteum* menurun secara signifikan ($p<0,05$) tetapi jumlah folikel Graafian dan *corpus luteum* meningkat secara signifikan dibandingkan dengan kelompok +VE Con ($p<0,05$). AA dapat meningkatkan apoptosis dan degradasi folikulogenesis jaringan ovarium dengan menurunkan ekspresi gen ERK. Pemberian NAC memperbaiki pengaruh buruk AA dalam dosis ketergantungan dan meningkatkan folikulogenesis dengan menurunkan tingkat apoptosis. Oleh karena itu, suplementasi NAC dapat memperbaiki tingkat kesuburan hewan.

Kata Kunci: Akrilamida, Apoptosis, ERK, Tikus Betina, N-asetilsisten

ABSTRACT

Naimi M, Shariati M, Naeimi S, Edalatmanesh MA. 2020. Effect of N-acetylcystein on ERK gene expression in ovarian tissue of acrylamide-treated adult rats. JITV 25(1): 11-18. DOI: http://dx.doi.org/10.14334/jitv.v25i1.2161.

Acrylamide (AA) is a toxic and carcinogenic compound produced in cooking process. The purpose of this study is to evaluate the effect of N-acetylcysteine (NAC) on extracellular signal-regulated kinase (ERK) gene expression level and ovarian histopathological changes in AA-treated rats. Thirty-six female adult Wistar rats were randomly divided into 6 groups including control, positive control (+VE Con), negative control (-VE Con), experimental 1 (Exp1), experimental 2 (Exp2) and experimental 3 (Exp3). Twenty eight days after the treatment, ERK gene expression level was measured by real-time PCR method and ovarian histopathological changes were evaluated. The ERK gene expression level was significantly decreased in the +VE Con, Exp1 and Exp2 groups as compared to the control group ($p<0.05$), but not in the -VE Con and Exp3 groups ($p>0.05$). Histologically, the +VE Con group showed a significant decrease in the number of primary, secondary and Graafian follicles as well as corpus luteum as compared to the control group ($p<0.05$), but not in the negative, Exp2 and Exp3 groups ($p>0.05$). In the Exp1 group, the number of primary and secondary follicles as well as corpus luteum significantly decreased ($p<0.05$), however, the numbers of Graafian follicle and the corpus luteum were significantly increased as compared to the +VE Con group ($p<0.05$). The AA was supposed to increase the apoptosis and folliculogenesis degradation in the rat ovarian tissue by decreasing ERK gene expression. Administration of NAC ameliorated the deleterious effects of AA in a dose-dependent manner and improve folliculogenesis by reducing apoptosis level. Thus, the NAC supplement could be helpful in ameliorating animal fertility.

Key Words: Acrylamide, Apoptosis, ERK, Female Rat, N-acetylcysteine

INTRODUCTION

Nowadays, with the change in lifestyles and diets, manufacturers are increasingly focused on fried foods, so that heating food at high temperatures is a common way of producing processed foods. Although heat processes are one of the most important ways for preserving foods that improve some food properties, it seems that these processes are capable of forming a wide range of toxic compounds such as acrylamide (AA) (Proietti et al. 2014). The AA has a chemical formula C_3H_5NO is a low-molecular-weight, colorless and odorless compound that is produced in foods containing starch (high amount) and protein (low amount) during cooking process at high temperatures (> 120 ˚C) and it is also widely used in industries and laboratories (Kahkeshani et al. 2014). Studies showed that AA is tumorigenic and carcinogenic in different human tissues (Klaunig 2008). Acrylamide is rapidly absorbed through the gastrointestinal tract and metabolized to glycidamide (GA) via cytochrome P450 2E1 monooxygenase, which is much more reactive than AA (Aydin 2018). Various studies confirmed the toxic effects of AA and its metabolite, GA, on neurons and the reproductive system as well as the carcinogenic effects on various tissues of the body such as the liver, kidney, intestine, and lung (Kahkeshani et al. 2014). Acrylamide can induce oxidative stress and reactive oxygen species by decreasing the capacity of the antioxidant system, thereby reducing glutathione levels and increasing lipids peroxidation (Zhang et al. 2011a). Active oxygen species play a key role in important biological processes such as cell apoptosis, meiosis restart, and aging. Increased levels of reactive oxygen species are associated with decreased intracellular oxidation-regeneration reactions, thereby increasing apoptosis (Duan et al. 2015). Although apoptosis is essential for the development and normal function of the ovary and testis, it has been found that AA can affect the reproductive system of both sexes by altering the expression of genes responsible for apoptosis (Duan et al. 2015; Camacho et al. 2012). The extracellular signal-regulated kinase (ERK) is a member of the mitogen-activating protein kinases family that controls a wide range of cellular activities such as proliferation, migration, differentiation, and death. Proper ERK activity induces the survival and inhibition of cell apoptosis and its overexpression causes cancer, but under certain conditions can also have a pro-apoptotic function (Cagnol & Chambard 2010; Sumizawa & Igisu 2007).

N-acetylcysteine (NAC), which is considered as a cheap and safe antioxidant and drug, is a potent source of sulfhydryl groups that converts to metabolites that stimulate glutathione production and directly removes free radicals (Mokhtari et al. 2017). The NAC has been used as a mucolytic drug in respiratory diseases for many years but has also been useful in the treatment of other diseases including cancer, heart diseases, heavy metal poisoning, influenza prevention, epilepsy and acetaminophen poisoning (Saha et al. 2013). Studies have shown that NAC can reduce the production of some cytokines such as alpha tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) and thus modulates pro-inflammatory pathways (Al-Shukaili et al. 2009). It has also been shown that NAC can inhibit apoptosis by decreasing oxidative stress and enhancing cell survival (Daneshpoya et al. 2017). Due to various pharmacological effects of NAC and its relative safety, the tendency to use it has increased in recent years (Demedts et al. 2005). This drug has an important functional spectrum in the body, such that it increases the cellular levels of reductive glutathione as a potent antioxidant system and on the other hand, improves the function and regulation of intracellular redox systems (Mokhtari et al. 2017; Markoutsa & Xu 2017). Also, immunological functions have been proposed (Arranz et al. 2008). Since ovarian tissue damage can lead to subfertility or infertility, and since AA can damage the reproductive system, especially ovarian tissue and considering that there has been limited research in this field, therefore, in the present study, the protective effect NAC on the ovarian tissue apoptosis was studied by measuring the expression level of ERK gene as well as the rate of morphometric and histopathological changes of ovarian tissue in AA-treated rats.

MATERIALS AND METHODS

Animals

Adult female Wistar rats weighing 220±20 g were obtained from the animals' house of Islamic Azad University of Kazerun and were adapted to the new environmental conditions for 2 weeks. They were kept at standard conditions at 22±2°C, 12 hours of darkness/daylight and 70% humidity. During the study, the animals had access to food and water *ad libitum*. The protocol of this study was approved by the Ethics Committee of Islamic Azad University of Kazerun, Iran, in relation to working with laboratory animal care (Ethical code no: IR.IAU.SHIRAZ.16330525651006).

The experiment protocol

The rats were randomly divided into 6 groups of 6 rats *i.e.* control, positive control (+VE Con), negative control (-VE Con), experimental 1 (Exp1), experimental 2 (Exp2) and experimental 3 (Exp3). The control group received no special treatment. Animals in the +VE Con

group received 50 mg/kg AA (Merck, Germany) orally. The animals in the -VE Con group received 40 mg/kg NAC (Merck, Germany) intraperitoneally. The animals in the Exp1, Exp2, and Exp3 groups received 10, 20 and 40 mg/kg NAC at 9 am intraperitoneally and 50 mg/kg AA at 5 pm orally. The study duration was 28 days and similar in all groups. The dose of AA and NAC was selected based on previous studies (Camacho et al. 2012; Chiew et al. 2016). At the end of the study, the animals were anesthetized using ether and the left and right ovaries of each animal were removed for histological examination.

Prior to the study, the rats co-cycling was performed to place them in a phase of the estrous cycle as the following. At first, 100 µg Estradiol valerate (Aburaihan, Iran) was dissolved in 0.2 ml olive oil and injected intramuscularly with an insulin syringe. After 42 hours, 50 µg progesterone (Aburaihan, Iran) was injected intramuscularly and 6 hours later, the vaginal smear was collected. Each stage of the estrous cycle was diagnosed based on the ratio between three types of cell populations (epithelial cells, keratinized cells, and leukocytes) observed in vaginal smears (Marcondes et al. 2002). Microscopic observations were made in rats that their estrous phase had been synchronized.

Quantitative analysis of ERK gene expression using real-time PCR

To extract RNA, a small piece of ovarian tissue was removed and washed twice with phosphate buffer saline. Then, the samples were evaporated and powdered in liquid nitrogen for 1 minute. Trizol (1 ml) (GeneAll Biotechnology, South Korea) was added to the resulting powder in a micro-tube and homogenized with a manual homogenizer for two minutes on the ice. The samples were incubated at room temperature for 5 minutes and then 200 µl chloroform was added for fat removal and shaken for 15 seconds. The resulting milky mixture was centrifuged at 12000 RCF for 15 minutes at 4°C. Then, the supernatant-containing RNA was transferred into a sterile micro-tube.

A volume of buffer RB1 (GeneAll Biotechnology, South Korea) was added to the micro-tube-containing supernatant and then the micro-tube contents were transferred to the column. After 30 seconds of centrifugation at room temperature at 12000 RCF, the infranatant was removed and 500 µl of buffer SW1 (GeneAll Biotechnology, South Korea) was added. After 30 seconds centrifugation at the room temperature and 12000RCF, the infranatant was removed again. Next, 500 µL of buffer RNW (GeneAll Biotechnology, South Korea) was added to the column, and after centrifugation at the room temperature and 12000 RCF, the columns were transferred to a new micro-tube and 50 µL of nuclease-free water was added and RNA was purified after centrifugation at 12000 RCF.

Using reverse transcription reaction (RT-PCR), mRNA conversion to cDNA was performed using the manufacturer's instructions (Biofact, South Korea). To investigate the quality of cDNA, the polymerase chain reaction with specific primer (Metabion, Germany; F-CGTGCGTGACATTAAAGAGAA and R-CGCTCATTGCCGATAGTGAT) for β-Actin gene as a housekeeping gene was examined. Then, in order to amplify the target fragment and quantify the expression of the ERK gene, Real-time PCR reaction was performed by a cyber green method using Qiagen-manufactured Q rotor Gene.

RT-PCR reaction using specific primers (Metabion, Germany; F- CGTTCAGATGTCGGTGTC and R-AAAGGAGTCAAGAGTGGG), ERK gene (Wang et al. 2015) and the reaction mixture of Power SYBR green PCR master mix (Applied Biosystems, UK) was performed during a program at 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds and at 60°C for 1 minute. The mean of CTs was calculated using $2^{-\Delta CT}$.

Histopathological analysis of ovarian tissue

After removing the left and right ovaries of all animals, the adipose tissue around them was removed and fixed in 10% buffered formalin. After tissue processing, the tissue samples were blocked in paraffin and then 5- micron thick serial sections were provided longitudinally and centrally using a microtome machine. The sectioned were stained with hematoxylin-eosin and then the number of primaries, secondary, Graafian follicles as well as corpus luteum was counted under light microscopy. The follicles were counted spirally from a point at the cortex clockwise toward the medulla.

Statistical analysis

SPSS software version 20 was used for data statistical analysis. At first, the normality of the data was confirmed using Kolmogorov-Smirnov test, and then by using one-way ANOVA and LSD post hoc tests, data analysis was performed at $p<0.05$. The results were expressed as mean ± standard deviation in the table and graph. The GraphPad Prism version 6 software (GraphPad Prism, Inc., San Diego, CA, USA) was also used to design ERK gene expression graph.

RESULTS AND DISCUSSION

Result

ERK gene expression

The results of ERK gene expression in different groups (Figure 1) showed that in the +VE Con group, the level of ERK gene expression had significantly decreased as compared to the control group ($p<0.05$), but no significant differences between the -VE Con and the control groups were observed ($p>0.05$). The gene expression level in the Exp1 and Exp2 groups were significantly decreased as compared to the control group ($p<0.05$), but in the Exp2 group, there was a significant increase in ERK gene expression level as compared to the +VE Con group ($p<0.05$). The Exp3 group had a significant increase compared to the +VE Con group ($p<0.05$), while there wasn't a significant difference with the control group ($p>0.05$).

Morphometric and histopathological findings of the ovarian tissue

Histopathological examination of the ovarian tissue in different groups (Table 1) showed that in the +VE Con group, the number of primaries, secondary and Graafian follicles as well as corpus luteum had significantly decreased as compared to the control group ($p<0.05$), while in the -VE Con group, no significant differences were found with the control group ($p>0.05$). In the Exp1 group, the number of primary and secondary follicles and corpus luteum decreased significantly ($p<0.05$). Also, the number of Graafian follicles and corpus luteum in the Exp1 group significantly increased compared to +VE Con group ($p<0.05$). There was no significant difference in the number of Graafian follicles in the Exp1 group ($p>0.05$). In the Exp2 and Exp3 groups, the number of primaries, secondary, Graafian follicles as well as corpus luteum increased significantly ($p<0.05$) compared to the +VE Con group ($p>0.05$), while there was no significant difference with the control group ($p>0.05$).

Morphometrically results in the ovarian tissue sections indicated that normal folliculogenesis did take place in the control, -VE Con, Exp2 and Exp3 groups and different follicles including Graafian follicles with a thick layer of granulosa cells and corpus luteum were present. In the +VE Con and Exp1 group, folliculogenesis had been largely destroyed and a small number of corpus luteum was observed indicating a decrease in ovulation (Figure 2).

Discussion

In this study, the effect of AA (50 mg/kg) and NAC (10, 20 and 40 mg/kg) on ERK gene expression levels, as well as morphometric and histopathological changes in the ovarian tissue in adult rats, was evaluated for 28 days. The results of this study showed that following AA administration, ERK gene expression level decreased. The ERKs are members of the family of mitogen-activated protein kinases and are commonly known as cell survival factors because they regulate cell proliferation and differentiation in response to growth factors. The apoptosis induced by growth factors depletion, cellular matrix detachment, and toxic factors may be suppressed by ERK activation. However, some studies have shown that some toxic agents such as AA can induce apoptosis and eventually cell death by involving ERK pathways (Sumizawa & Igisu 2007). The ERK pathway is associated with classic apoptosis markers such as caspase-3 and annexin V. Depending on the cell type and the nature of the injury, the ERK pathway activity can release cytochrome *c* from the mitochondria and activate caspase-9 inhibitor or activates caspase-8. The activity of ERK pathway can also increase the level of death ligands such as Fas, FasL and TNF-α. The ERK pathway can induce Fas activation, thereby inducing caspase-8 activation and inducing the apoptosis process. However, it seems that ERK-mediated caspase-8 activation can occur independently from Fas activation (Cagnol & Chambard 2010). It has been shown that the expression level of pro-apoptotic genes such as Fas and Caspase-3 can be increased in the testes of AA-treated rats, which may be related to the ERK pathway (Camacho et al. 2012; Sumizawa & Igisu 2007). According to the results of this study, it seems that decreased ERK expression level could be related to the increased Fas expression level, thus ERK-induced apoptosis can be mediated through Fas and increase the level of apoptosis in ovarian tissue cells due to AA administration.

In the present study, AA administration reduced the number of primary, secondary graafian follicles as well as corpus luteum. The studies indicate that the toxic effects of AA on the female reproductive system decrease ovarian weight and the number of mature oocytes (Duan et al. 2015). Acrylamide can also have a negative effect on the ovarian-follicle development and corpus-luteum formation (Wei et al. 2014), which is consistent with the results of this study. Acrylamide may induce apoptosis in oocytes by increasing the reactive oxygen species and changing redox reactions. Previous studies have shown that AA induces apoptosis among oocytes by increasing Annexin V. Annexin V is a phospholipid-binding protein activates during the early stages of apoptosis (Duan et al. 2015). Following

Table 1. Comparison the number of primary, secondary, Graaf and corpus luteum (mean ± SD) in different groups.

Groups	Parameters			
	Primary Follicle	Secondary Follicle	Graafian Follicle	Corpus Luteum
Control	22.66±3.01	7±1.67	3.33±1.21	11.50±1.04
+VE Con	15±1.26*	4±1.41*	1.83±0.4*	5.83±1.16*
-VE Con	22.33±2.16†	6.83±1.47†	3.16±0.75†	11±1.26*†
Exp1	16.50±1.87*	4.50±1.37*	3±0.63†	7.50±1.87*
Exp2	20.16±1.94†	5.83±1.47†	3.16±1.32†	10.16±1.16†
Exp3	21±2.19†	6.50±1.87†	3.33±0.51†	10.33±0.51†

* Significantly different ($p<0.05$) as compared to the control group.† Significantly different ($p<0.05$) as compared to the +VE Con group

AA administration in pregnant guinea pigs, the number of healthy follicles decreased and the number of apoptotic cells increased. It appears that AA is able to break down cumulus-oocyte cellular junctions by destroying vimentin filaments and eventually induces apoptosis (Hułas-Stasiak et al. 2013). The reduction of ovarian follicles and corpus luteum may also be due to the negative effect of AA on the nitric oxide synthase-dependent signaling pathway (Wei et al. 2014). Acrylamide can target and inhibit some of the cytoskeletal motor proteins such as Dynein and Kinesin. These proteins integrate cytoskeletal elements such as intermediate filaments, microfilaments, and microtubules into functional units. The inhibition of these proteins destroys the cytoskeleton, which subsequently affects cell-cell adhesion, cellular shape, intracellular communication, metabolism, synthesis and bio-chemicals' secretion (Camacho et al. 2012). Therefore, it seems that AA can disrupt the development of ovarian follicles and corpus luteum by targeting the cytoskeletal system.

N-acetylcystein is a low-molecular-weight thiol, which is derived from the amino acid cysteine and acts as a glutathione precursor, the main antioxidant in the body and increases its amount. N-acetylcystein plays an important role in reducing oxidative stress and eliminating reactive oxygen species and nitrogen due to its antioxidant and anti-inflammatory activities (Galhardo et al. 2007). It seems that the administration of NAC can counteract this decrease during the oxidative stresses that decrease glutathione levels and act as an antioxidant as well as an antitoxin by increasing glutathione levels. It is therefore expected that NAC will be used in a variety of diseases (Altinoz et al. 2015; Millea 2009). Glutathione plays an important role in neutralizing the pathway of toxins, including peroxide compounds and other molecules that produce free radicals and therefore has a potent protective effect on cells (Alturfan et al. 2012). NAC can also modulate pathophysiological processes such as oxidative stress, neurodegenerative processes, apoptosis and mitochondrial dysfunction (Mokhtari et al. 2017). According to the results of this study, the administration of NAC in AA-treated rats in experimental groups increased ERK gene expression in a dose-dependent manner, however, NAC administration alone in the –VE Con group did not change the level of ERK gene expression. N-acetylcystein has been shown to play an important role in chondrocyte survival by inhibiting inflammatory factors and activating ERK expression levels (Li et al. 2000). The evidence also suggests that NAC may play a role in down-regulating Fas gene expression. N-acetylcystein can reduce the sensitivity of the cells to Fas-mediated apoptosis by modulating the activity of the enzyme responsible for Fas cleavage (Delneste et al. 1996). Although the protective effects of NAC may be due to its ability as a glutathione precursor, it has been suggested that the antioxidant capacity of NAC can act independently of glutathione and play a protective role in the induced toxicity. The NAC functions in enhancing cell survival can be directly and indirectly due to its antioxidant capacity and the ability to remove free radicals (Zhang et al. 2011b).

Based on our observations, the administration of NAC in AA-treated rats in a dose-dependent manner significantly increased the number of primaries, secondary and Graafian follicles as well as corpus luteum compared to the +VE Con group, but the administration of corpus luteum alone in the -VE Con

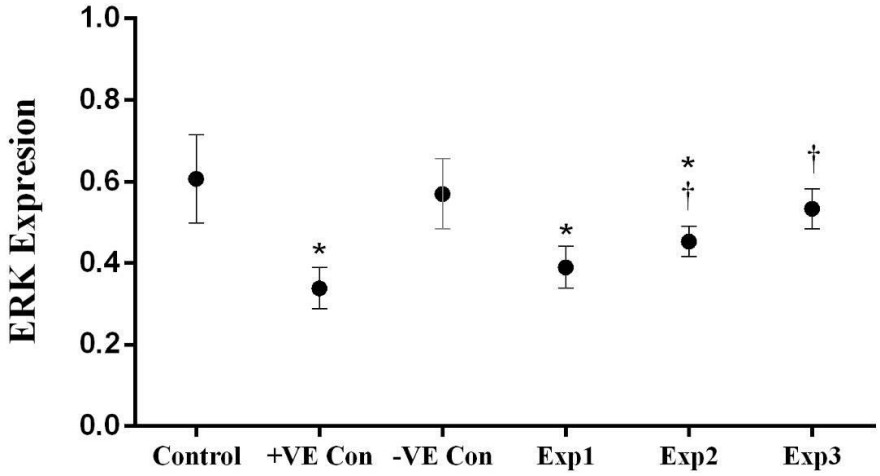

Figure 1. Comparison of ERK gene expression levels (mean ± SD) in different groups. * Significantly different (P<0.05) as compared to the control group. † Significantly different (P<0.05) as compared to the +VE Con group.

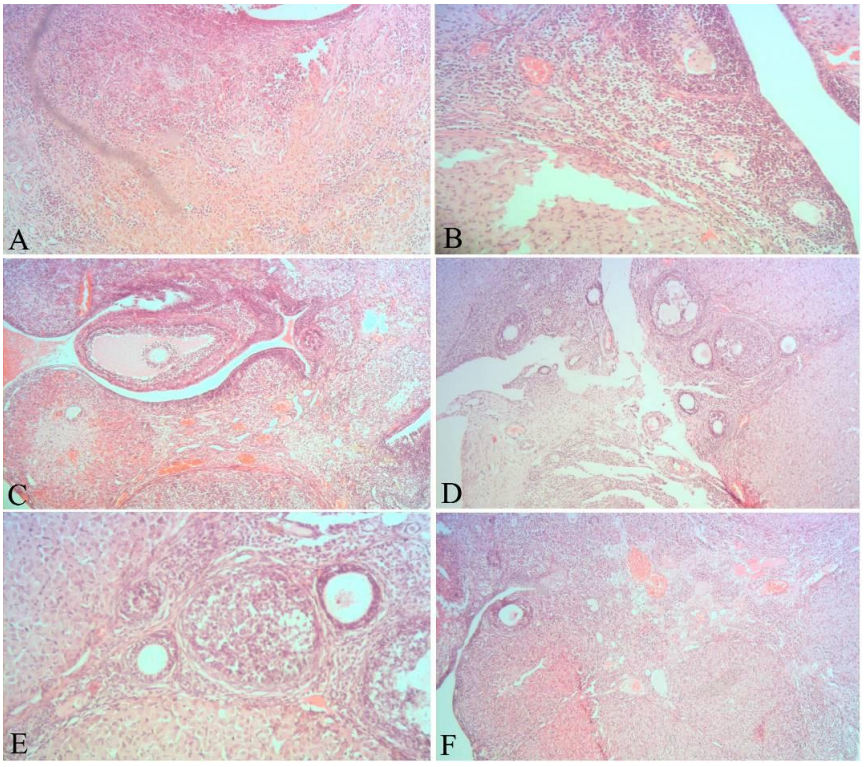

Figure 2. Photomicrograph of ovarian tissue in the rats treated with AA and NAC in different groups. A) Normal folliculogenesis was observed in the control group. B) In the +VE Con group, the reduction of ovarian follicles and corpus luteum is observed. Folliculogenesis was deteriorated. C) In the -VE Con group, the Graafian follicle is observed with a thick layer of granulosa. D) A small number of Graafian follicle and corpus luteum was observed in the experimental group 1. E and F) In the Exp2 and Exp3 groups, ovarian follicles are seen along with the corpus luteum. Folliculogenesis ameliorated. H&E staining. A, C, D and F 4X magnification. B and E 10X magnification.

group did not change the number of ovarian follicles and corpus luteum.

Although in our study, the administration of NAC alone did not affect on the ovarian tissue, it has been reported that during the ovarian aging process, appropriate treatment with NAC delays apoptosis and the death of healthy follicles and since oocytes and follicles can be affected by oxidative stress in the body, the ovary can be a suitable target for NAC activity (Liu et al. 2012). Nitric oxide has an important role in the development of ovarian follicles, oocyte development, ovulation, luteinization, and steroidogenesis. Nitric oxide also plays a key role in the modulation of oxidative stress and because it can directly react with Heme proteins, its excessive increase can be caused by reacting with reactive oxygen species and nitrogen, causing ovary dysfunction. Nitric oxide synthesis in the ovary is also regulated by cytokines (Hattori & Tabata 2006). Acrylamide has been shown to increase the expression of nitric oxide (Lyn-Cook et al. 2011). Nitric oxide is synthesized by nitric oxide synthase, and AA can exert its cytotoxic effects through the nitric oxide synthase pathway on the development of ovarian follicles (Wei et al. 2014). NAC appears to modulate the toxic effects of AA on the development of ovarian follicles by affecting nitric oxide activity (Xia et al. 2006).

CONCLUSION

Acrylamide at a dose of 50 mg/kg had toxic effects on the ovarian tissue of female rats for 28 days, thereby reduced ERK gene expression and apoptosis. Acrylamide administration affects the ovarian follicles and destroys folliculogenesis. However, NAC administration in female rats treated with AA could counteract the toxic effects of AA by increasing the expression of ERK gene, reducing apoptosis and improve folliculogenesis in a dose-dependent manner. Therefore, NAC seems to be effective in reducing the negative effects of AA on the ovarian tissue of adult rats.

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Effect of Mix Culture Bacteria and Fungi in Fermented Peanut Hulls-Based Feed Supplement on Physical Quality and *In vitro* Rumen Fermentation Parameters

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ABSTRAK

Nesti DR, Baidlowi A, Fauzi A, Tjahajati I. 2020. Pengaruh kombinasi campuran bakteri dan jamur pada kualitas fisik fermentasi pakan suplemen berbasis kulit kacang terhadap kualitas fisik dan parameter fermentasi rumen secara *in vitro* . JITV 25(1): 19-25. DOI: http://dx.doi.org/10.14334/jitv.v25i1.2079.

Penelitian ini bertujuan untuk mengetahui pengaruh kombinasi campuran bakteri (EM4:E) dan jamur (*Trichoderma viridae:*TV) sebagai inokulan pada fermentasi pakan suplemen berbasis kulit kacang terhadap kualitas fisik dan parameter fermentasi rumen secara *in vitro*. Pakan Basal (PB) dibagi kedalam empat perlakuan, yaitu: P_0 (PB); P_1 (E:25%+TV:75%); P_2 (E:50%+TV:50%); dan P_3 (E:75%+TV:25%), setiap perlakuan menggunakan tiga kali ulangan. Pakan difermentasi selama sembilan hari secara fakultatif anaerob. Parameter yang diamati meliputi kualitas fisik pakan (warna, aroma, kenampakan jamur, dan pH) serta parameter fermentasi rumen secara *in vitro* (pH rumen, kadar protein mikrobia, dan kadar total asam lemak terbang). Data dianalisis dengan menggunakan rancangan acak lengkap pola searah (*One-way* ANOVA), jika terdapat perbedaan nyata dilanjutkan dengan uji *Kruskall-Wallis* untuk data kualitas fisik dan uji *Duncan's New Multiple Range Test* (DMRT) untuk data parameter fermentasi dalam rumen secara *in vitro*. Hasil penelitian menunjukkan bahwa kombinasi E dan TV dengan perbandingan yang berbeda tidak mempengaruhi parameter aroma dan kenampakan jamur pada parameter kualitas fisik ($P \geq 0,05$), tetapi berpengaruh nyata ($P \leq 0,05$) terhadap perubahan warna dari coklat kehitaman (1,64) pada P_0 menjadi warna kecoklatan pada P_1, P_2 dan P_3 berturut-turut: 2,44; 2,69; dan 2,80, serta penurunan pH berturut-turut: 10,67; 10,67 dan 10,67%, dari P_0. Kombinasi inokulum dengan perbandingan yang berbeda tidak memberikan pengaruh nyata ($P \geq 0,05$) terhadap parameter pH rumen, kadar protein mikrobia dan kadar total VFA. Berdasarkan hasil penelitian dapat disimpulkan bahwa fermentasi suplemen pakan berbasis kulit kacang dengan kombinasi EM_4® 25%:*Trichoderma viridae* 75% memberikan hasil terbaik dalam parameter kualitas fisik pakan fermentasi ditinjau dari warna serta pH dengan tidak menggangu parameter fermentasi dalam rumen.

Kata Kunci: EM_4®, Fermentasi Rumen secara *In vitro*, Kulit Kacang Tanah, Kualitas Fisik, *Trichoderma viridae*

ABSTRACT

Nesti DR, Baidlowi A, Fauzi A, Tjahajati I. 2020. Effect of Mix Culture Bacteria and Fungi in Fermented Peanut Hulls-Based Feed Supplement on Physical Quality and *In vitro* Rumen Fermentation Parameters. JITV 25(1):19-25. DOI: http://dx.doi.org/10.14334/jitv.v25i1.2079.

The purpose of this research was to determine the effect of the combination of mix culture bacteria (EM4®:E) and fungi (*Trichoderma viridae*:TV) on physical quality and *in vitro* rumen fermentation parameters of peanut hull-based feed supplements. Basal feed was divided into four treatments, which were: P0 (BF); P1 (E:25%+TV:75%); P2 (E:50%+TV:50%),; and P3 (E:75%+TV:25%); and each treatment had three replications. Feeds were fermented facultative anaerobically for nine days. The observed parameters were physical qualities (color, odor, fungi appearance and pH), and *in vitro* rumen fermentation parameters (rumen pH, microbial protein content, and total volatile fatty acid content). Data were analyzed using one way ANOVA design, and the significance of differences were tested using Kruskall-Wallis test for the physical qualities data and Duncan's New Multiple Range Test (DMRT) test for *in vitro* rumen fermentation parameters. Results showed that the combination of E and TV at different level did not affect odor and the fungi appearance parameters ($P \geq 0.05$), however, it significantly affected $P \leq 0.05$) color change from blackish (1.64) in P0 to brownish in P1, P2 and P3 of 2.44; 2.69; and 2.80, respectively. The pH also decreased significantly ($P \leq 0.05$) by 10.67%. Treatment also did not affect the rumen pH, microbial protein content, and total volatile fatty acid content ($P \geq 0.05$). It is concluded that the combination of the EM4® 25%: *Trichoderma viridae* 75% on peanut-hull based feed supplement fermentation gives the best result on color and pH fermented feed product without affecting the rumen fermentation process.

Key Words: EM_4®, *In vitro* Rumen Fermentation, Peanut Hull, Physical Quality, *Trichoderma viridae*

INTRODUCTION

In Indonesia, the availability of forages as feed sources for ruminants during the dry season is very limited, so the role of agriculture-based waste or by-products was very important as an alternative feed source. One of the potential feedstuffs from agriculture wastes is the peanut hulls. According to the Kementerian Pertanian Republik Indonesia (2019), Indonesia might produce approximately 512,198 tons of peanuts. The problem of peanut hulls as a feed ingredient is that it contains relatively high crude fiber (61.99%), and low (8.74%) crude protein (Fauzi 2019), which limits its digestibility (Tillman et al. 1991). Technology intervention could be used to improve the nutrient contents of peanut hull, such as supplementation with other feedstuffs or nutrients and through the fermentation process (Natsir 2012). Feed supplements can support the development of rumen microbes and improve feed digestibility (Wina 2005; Pamungkas et al. 2008), while fermentation process might improve the feed quality, due to the involvement of microbes in digesting and fermenting fiber (cellulose, hemicellulose) and anti-nutrient compounds (Rokhmani 2005).

Trichoderma viridae is a fungus which produce cellulolytic enzyme, which degrades complex polysaccharides into simple polysaccharides and improve the fiber digestibility (Jaelani et al. 2015). Rizali et al. (2018) showed in their research that the utilization of *Trichoderma viride* on the fermentation process of palm oil midrib and leaves might decrease crude fiber content by up to 8.81%. EM4® is a mix culture of bacteria, consisting mainly of five genus: *Rhodopseudomonas* sp., *Lactobacillus* sp., *Streptomyces* sp., *Actinomycetes*, and *Saccharomices* sp (Casmia 2016; Santoso & Aryani 2007); Satria & Nurhasanah 2010), that capable of degrading crude fiber and improve its digestibility (Ismanto 2018). Telew et al. (2013) showed in their research that EM4 decreased crude fiber content of rice hulls up to 24%. Lokapirnasari et al. (2015) explained in their study using the combination of bacteria *Acidothermus cellulolyticus* and *Aspergillus terreus* might decrease crude fiber content on rice bran fermentation.

According to the potential of peanut-hull and the bacteria and fungi as an inoculant source for feed fermentation, this research was conducted to evaluate the effects of combinations of mix culture bacteria and fungi on the physical and fermentation characteristics of peanut hull.

MATERIALS AND METHODS

Feed preparation and formulation

Peanut hull as the main feedstuff for this research is obtained from local farmers in Kulon Progo Regency, Yogyakarta Province, Indonesia. Other feedstuffs are obtained from PT. Hasta Karya Damai Manunggal, Boyolali Regency, Central Java, Indonesia. Peanut hull was finely ground and then mixed with other ingredients such as: palm kernel meal, copra meal, pollard, corn gluten meal, minerals, molasses and urea. The feed formulation and nutrient contents is shown in Table 1. *Trichoderma viride* was obtained from the Pusat Antar Universitas (PAU), Universitas Gadjah Mada and EM4® was obtained from Sleman Regency Yogyakarta Province, Indonesia.

Inoculum preparation

Trichoderma viridae (pure) was cultured using Potato Dextrose Agar (PDA) media and re-cultured using Potato Dextrose Broth (PDB) media. Cultures were stored for 5 days at 37°C incubators, then counted using a spectrophotometer on 600nm to find out the fungal cells count. The use of the fungi inoculant on this research is on the liquid state. The cell count of the solution EM4® was counted using a spectrophotometer on 600nm (Syauqi 2017). The use of the EM4 is prepared directly when ready to use in.

Feed supplements fermentation and treatment groups

Water content (WC) of formulated feed supplements was adjusted to 60% using a mix of water and inoculums. 2% of inoculum on the liquid state, from total water-used to produce 60% WC is mixed with subtracted water-inoculum water. Each treatment was weighed (250 g) and was fermented using 2 kg plastic bags silos. Fermented feed supplements were divided into four groups of treatments, in three replications:

P_0: Basal feed
P_1: P_0 + *Trichoderma viridae* (75%) + EM_4® (25%)
P_2: P_0 + *Trichoderma viridae* (50%) + EM_4® (50%)
P_3: P_0 + *Trichoderma viridae* (25%) + EM_4® (75%)

Oxygen was sucked from the silo using *vacuum* tools. Silos already in anaerobic conditions were then inserted with the needle in a loose plastic section that was closed with cotton in order to form a facultative anaerobic condition. The fermentation process lasted for 9 days (Mirwan 2018).

Physical quality test

The physical qualities observed in this study were color, odor, fungi appearance and pH base on (Irawati et al. 2019). The determination of the physical quality of feed supplements was conducted by 15 reliable panelists. Feed fermentation results were taken as much as 50 grams and were placed in a small cup. Tests were conducted based on the scoring and then the data obtained was transformed into a numerical scale, as follows:

Table 1. Formulation and nutrient contents of experimental feedstuffs

Feedstuff	Formulation (%)	DM (%)	CP (%)	CF (%)	EE (%)
Peanut hull[1]	50	49.63	4.37	30.9	0.29
Pollard[2]	5	4.30	0.82	0.29	0.20
Palm kernel cake[2]	15	12.90	2.12	1.61	1.79
Copra cake[2]	12	10.32	3.31	0.82	1.35
CGF [3]	10	8.60	2.38	0.67	0.20
Urea[3]	1	0.86	2.88	0.00	0.00
Molasses[3]	5	3.85	0.20	0.02	0.02
Minerals [3]	2	1.72	0.00	0.00	0.00
Total	100	92.18	10.8	35.7	5.87

[1]Fauzi (2019), [2]Hartadi et al. (2017), [3]NRC (2001)
DM = Dry matter, CP = Crude protein, CF = Crude fiber, EE = Ether Extract

Table 2. Laboratory analysis of nutrient contents of experimental fermented feed

Groups	DM (%)	CP (%)	CF (%)	NFE (%)
P_0	96.52	16.12	33.48	39.45
P_1	95.50	16.12	39.17	33.90
P_2	95.50	15.98	38.41	34.27
P_3	95.53	15.73	38.31	34.68

P_0=Basal feed, P_1= P_0 + *Trichoderma viride* (75%) + EM4® (25%), P= P_0 + *Trichoderma viride* (50%) + EM4® (50%), P_3= P_0 + *Trichoderma viride* (25%) + EM4® (75%), DM = Dry matter, CP = Crude protein, CF = Crude fiber, NFE = Nitrogen Free Extract

1. Color: Score 1=blackish brown; score 2=chocolate; score 3=brownish yellow; score 4=yellow.
2. Odor: Score 1=very bad; score 2=rather rotten; score 3=slightly acidic; score 4 = acid.
3. Pathogenic fungi appearance: Score 1=lots of fungi; Score 2=middle fungi; score 3=no fungi

Fermented feed product pH

One gram of fermented feed was diluted with nine ml of *aquadest* and pH was determined using pH Meter (AOAC 2006).

***In vitro* rumen fermentation**

In vitro rumen fermentation using the method of Menke & Steingass (1988) as modified by Makkar et al. (1995). Samples (300 mg) were weighed and mixed with 30 ml rumen: buffer solution (1:2) (vol/vol) and incubated for 48 hours. Gas production was measured at 0; 2; 4; 6; 8; 12; 24; and 48 incubation hours. After 48 hours of incubation, samples were separated between filtrate and the supernatant by filtration. The filtrate used for rumen pH was pH Meter (Kim et al. 2019), rumen microbial protein using Lowry's method (Plummer 1978), and VFA determination using gas chromatography (Filípek & Dvořák 2009).

Statistical analysis

The experiment was arranged in a *Completely Randomized Design* and data were analyzed using the ANOVA. Data were processed using the SPSS version 16.0 program. The significance of difference among the treatment means was tested using Kruskall-Wallis test for the physical qualities data and the Duncan's New Multiple Range Test (DMRT) test for *in vitro* rumen fermentation parameters.

RESULTS AND DISCUSSION

Physical quality

Effect of the combinations of EM4® and *Trichoderma viridae* fungi in fermented peanut hulls-based feed supplements on the physical quality and fermented product's pH is shown in Table 3.

The inoculum combination of EM4® and *Trichoderma viridae* fungi in fermented peanut hulls-based feed significantly changed ($P \leq 0.05$) the

fermented feed color value parameter between P_0 group (brown) and P_1, P_2 and P_3 groups (yellowish). The color change might be caused by microorganism's metabolism which degraded organic matter in the feed during the fermentation process. Microorganism's fermentation produced heat during the process that might affect the color of the fermented products. Munawaroh & Anggraini (2017) reported that the change in color of fermentation treatment was caused not only by the influence of temperature during the fermentation process but also by the type of raw material. Yunianta & Hartatik (2015) cited from Sianipar & Simanihuruk (2009), explained in their study that the silage without inoculum might have a darker color because the living in the early phase of active aerobic respiration, degradation of organic matter produces water, CO_2 and heat and the increasing temperature might affect the color of the silage.

The addition of different level combinations of EM4® and *Trichoderma viridae* fungi in the peanut hulls-based fermented feed did not change the odor parameter ($P \geq 0.05$). The results showed that the average value of the odor of supplement food in each treatment has a slightly acidic interpretation. This slight acidic odor might come from substrates degradation by the microorganism. The P_1, P_2, and P_3 groups showed higher acidic odor value compared to the P_0 group. The addition of inoculum may have caused changes in carbohydrates and their derivative compounds to alcohol, acids, and carbon dioxide. Munawaroh et al. (2015) reported in her study that during the fermentation process there is a conversion of glucose to pyruvic acid and then to lactic acid. The production of these lactic acids might cause the aroma of fermentation to become acidic.

The addition of different level combinations of EM4® and *Trichoderma viridae* fungi in the peanut hulls-based fermented feed did not show any differences in the pathogenic fungi appearance parameter ($P \geq 0.05$). This could happen because *Trichoderma viridae* fungi used the substrates and prevent the usage of it by other pathogenic fungi. It could be proven by the change of the color and odor parameter. Yunianta & Hartatik (2015) reported that the *Trichoderma* fungi appearance is significantly risen linearly with the fermentation time on corn straw fermentation using *Trichoderma* fungi. *Trichoderma sp*. might suppress the growth of pathogenic fungi by the antibiosis process, competition on substrates usage with the other pathogens fungi, and its hyper-parasitic traits which will lyse other pathogens fungi's hyphae (Purwantisari & Hastuti 2009).

The addition of different level combinations of EM4® and *Trichoderma viridae* resulted in the differences in pH between the P_0 groups ($P \leq 0.05$) and P_1, P_2, and P_3 (Table 4). It might occur because both inoculums have the ability to degrade organic substrates and produce lactic acid that decreased the pH of the

Table 3. The effect of the combinations of EM4® and *Trichoderma viridae* fungi in fermented peanut hulls-based feed supplement on the physical quality and fermented product's pH

Parameter	Treatment			
	P_0	P_1	P_2	P3
Color*	1.64[a]	2.44[b]	2.69[b]	2.80[b]
Odor(ns)	2.98	3.22	3.29	3.36
Fungi(ns)	3.00	3.00	3.00	3.00
pH*	7,5[a]	6,7[b]	6,7[b]	6,7[b]

P_0=Basal feed, P_1= P_0 + *Trichoderma viride* (75%) + EM4® (25%), P= P_0 + *Trichoderma viride* (50%) + EM4® (50%), P_3= P_0 + *Trichoderma viride* (25%) + EM4® (75%)

Table 4. The effect of the combination of EM4® and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed supplement to *in vitro* rumen parameters

Parameter	Treatment			
	P0	P1	P2	P3
pH[ns]	6,76	6,74	6,77	6,73
Rumen Microbial protein (mg/100ml)[ns]	8,97	9,37	11,54	10,19
Total VFA[ns]	118,13	107,81	126,27	131,22

P_0=Basal feed, P_1= P_0 + *Trichoderma viride* (75%) + EM4® (25%), P= P_0 + *Trichoderma viride* (50%) + EM4® (50%), P_3= P_0 + *Trichoderma viride* (25%) + EM4® (75%), [ns]= non-significant, VFA= Volatile Fatty Acid

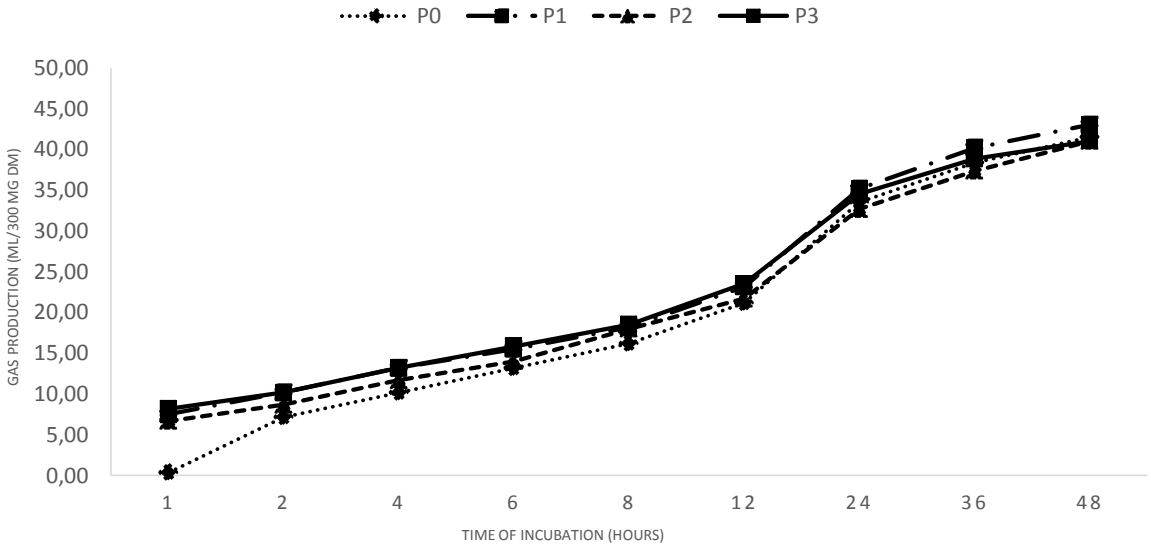

Figure 1. *In vitro* Gas production value of four different groups of the combination of EM4® and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed supplement: P_0 = Basal Feed; P_1 = P_0 + *Trichoderma viride* (75%) + EM4® (25%); P_2 = P_0 + *Trichoderma viride* (50%) + EM4® (50%); and P_3 = P_0 + *Trichoderma viride* (25%) + EM4® (75%).

fermented product. The optimal pH for the growth of *Lactobacillus* bacteria, which one of the bacteria on EM4, was 6,8 (Pramono et al. 2003) and *Trichoderma viridae* was 6,2 (Juliana et al. 2017). Zakaria et al. (2013) stated that the more colonies of lactic acid bacteria grew in the fermentation process, the fermentation process will result in lower pH. *Trichoderma viridae* fungi produce alcohol during the fermentation process while EM_4® produced lactic acid as a by-product of the fermentation process (Umrah et al. 2009). Wahono et al. (2011) reported that in their research using EM_4®: organic acids like acetic acid, pyruvic acid, and lactic acid decreased pH strongly, while other acids, such as butyric acid and other fatty acids had only a slight effect decrease in pH.

In vitro fermentation

In vitro gas production value of four different groups of the combination of EM4® and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed supplement are shown in Figure 1. Gas production illustrates the activity of microbes in degrading feed in the rumen. The results showed that incubation time-correlated with gas production and there was no significant difference ($P \geq 0.05$) between P0, P1 P2 and P3 groups on *in vitro* gas production. The gas production might not significantly different because the formulation, feed ingredients and nutritional content between the groups are slightly the same. Microbial rumen can ferment all types of feed ingredients and the role of the microbes in ruminants was very important because 65% of the main feed of ruminants is degraded by rumen microbes (Firsoni & Lisanti 2017). Abo-Donia et al. (2014) presented in his research that fungal using *Trichoderma viride* and urea might increase the gas production of peanut hull *in vitro* parameter because *Trichoderma* and urea might loosen the *ligno-cellullose* bonds and increase the solubility of hemicellulose and the soluble fraction.

The effect of the combination of EM4® and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed supplement to *in vitro* rumen parameters is showed in Table 3. The result showed that the addition of a different level combination of EM4® and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed did not show any differences in rumen pH after *in vitro* test ($P \geq 0.05$). The rumen pH is at normal condition indicating that the fermented feed did not affect the rumen pH condition, even though the fermented pH is slightly acidic. The pH of the feed product did not affect the rumen pH because it's on the normal range of rumen pH, which is 6.0-7.0. Mardalena (2015) stated that the normal pH rumen was a key factor for optimal rumen function. The change in rumen pH will affect the microbial population and further affect the digestibility of feed.

Rumen microbial protein content was not affected by the addition of a different level combination of EM4® and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed ($P \geq 0.05$). It could occur because of the supply rate of carbohydrate and nitrogen amount. The crude protein content on the fermented peanut hull feed using EM4 and *Trichoderma* fungi might play the role of the rumen microbial protein content. Abo-Donia et al. (2014) explained that

fermentation using fungi treatment might decrease NH_3-N release, and carbohydrate was degraded to provide energy for microbial multiplication resulting microbial mass and N-balance increase.

Total VFA content was not affected by the addition of different level combinations of EM4® and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed ($P \geq 0.05$). McDonald et al. (2002) stated that the average normal VFA production was 70-150 mM, while in this study the total VFA produced ranged from 107.79 to 135.30 mM. It may be related to a similar amount of complex carbohydrate and protein substrates from fermented feed used by rumen microbes in rumen fermentation. Nurjana et al. (2015) explained that the ensiling process using *Trichoderma reesei* on napier grass did not affect the VFA content due to the high content of low digestible saccharides and crude protein in the napier grass fermented product.

CONCLUSION

The inoculum combination of EM4® and *Trichoderma viridae* fungi in fermented peanut hulls-based feed supplement gives accepted physical quality of fermented feed product. Color, odor, and fungi appearance met the requirements of good fermented feed. It also gives no negative effect on *in vitro* rumen fermentation parameters such as pH, rumen microbial protein content and total VFA content. However the combination of *Trichoderma viridae* (75%) + EM_4® (25%) gives the best result in color and pH parameters while it does not affect the rumen pH, rumen microbial and VFA parameters.

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Effects of Two Different Energy Sources in Total Mixed Diets on the Performances and Blood Metabolites of Lactating Boerka Goats

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ABSTRAK

Ginting SP, Tarigan A, Simanihuruk S, Antonius, Solehuddin. 2020. Pengaruh dua sumber energi berbeda dalam pakan komplit terhadap performans dan metabolit darah kambing Boerka laktasi. JITV 25(1): 26-33. DOI: http://dx.doi.org/10.14334/jitv.v25i1.2196.

Kambing dalam masa laktasi rentan terhadap situasi neraca energi tubuh yang negatif akibat mobilisasi cadangan lemak tubuh untuk memenuhi kebutuhan energi yang meningkat untuk produksi susu. Penelitian bertujuan untuk mempelajari respons kambing fase laktasi terhadap dua sumber energi berbeda didalam pakan komplit. Penelitian dilakukan menggunakan 35 ekor kambing Boerka dengan paritas 2-3, bobot badan 30,3±4,48 kg dan skor kondisi tubuh 2,5±0,05 skala 1-5 pada saat melahirkan. Ternak dialokasikan ke lima perlakuan pakan (7 ekor/perlakuan) yang dirancang sedemikian rupa, sehingga iso-nitrogen dan iso-kalori. Tepung kasava digunakan sebagai sumber utama energi glukogenik dan bergafat digunakan sebagai sumber utama energi lipogenik. Pakan diberikan dalam bentuk pakan komplit pelet.Tidak terdapat perbedaan konsumsi pakan antara pakan glukogenik (rendah lemak 1,40 dan 2,28%), namun berbeda nyata (P<0,05) dengan kelompok pakan lipogenik (kandungan lemak pakan 4,7-7,5%). Selama laktasi kambing mengalami pertambahan berat badan pada kisaran 15-46 g/h yang mengindikasikan neraca energi positif pada semua perlakuan pakan. Kelompok dengan pakan energi lipogenik mengalami pertambahan berat badan, FCR dan skor kondisi tubuh yang lebih baik (P<0,05) dibandingkan dengan pakan dengan energi yang lebih glukogenik. Konsentrasi glukosa dan urea darah tidak dipengaruhi oleh perlakuan pakan (P>0,05), dan berturut-turut berkisar antara 37 - 43 mg/dl dan 39 to 51 mg/dl. Namun, secara numerik konsentrasi glukosa dan urea darah meningkat secara linier pada kelompok yang mendapat pakan lipogenik yang konsisten dengan taraf konsumsi pakan. Disimpulkan bahwa pemberian pakan dengan sumber energi lipogenik (kandungan lemak 7,5%) dalam bentuk pakan komplit bentuk pelet selama masa laktasi pada kambing menghasilkan taraf konsumsi pakan dan konsumsi nutrien dan PBB serta skor kondisi tubuh yang lebih tinggi dibandingkan dengan pemberian pakan dengan sumber energi glukogenik.

Kata Kunci: Pakan Glukogenik, Performa Kambing, Laktasi, Pakan Lipogenik

ABSTRACT

Ginting SP, Tarigan A, Simanihuruk S, Antonius, Solehuddin. 2020. Effects of two different energy sources in total mixed diets on the performances and blood metabolites of lactating Boerka goats. JITV 25(1): 26-33. DOI: http://dx.doi.org/10.14334/jitv.v25i1.2196.

Lactating goats are prone to negative energy status due to increased body fat reserve mobilization to support the high energy requirement of milk production. The study was aimed to investigate the responses of the lactating goat on diets provided in total-mixed ration differing in the energy sources. The experiment was conducted using a total of 35 does having 2-3 parities and an average bodyweight of 30.3±4.48 kg and BCS of 2.5 ± 0.05 on a scale basis of 1 to 5. Animals were allocated to one of five dietary treatments (seven animals/treatment) formulated to be iso-nitrogen dan iso-calory in a total mixed ration. Cassava meal was used as the source of glucogenic energy and bergafat as the main source of lipogenic energy. There were no DM intake differences (P>0.05) between the glucogenic diet (1.49% and 2.28% fat), but significant increases (P<0.05) in DM intake were observed in goats fed lipogenic diets (fat content range from 4.7 to 7.5%). All animals gained during the lactation period with ADG ranged from 15 to 46 g, indicating that all experimental animals were in positive energy balances. Goat receiving more glucogenic diets gained least and having higher FCR compared to those receiving lipogenic diet (P<0.05). Body condition scores were also improved in lactating goat received more lipogenic diets. Blood glucose and blood urea concentration was not affected by diet treatments and lactation period (P>0.05) and ranged from 37 to 43 mg/dl and 39 to 51 mg/dl, respectively. Numerically, however, the blood glucose and urea level linearly increased as the diet becoming more lipogenic due to the increased feed intake. It is concluded that lactating goats offered diets with lipogenic energy sources (7.5% fat content) presented in pelleted total mixed-ration during the entire lactation period had a higher dry matter and nutrient intakes, body weight gain and body condition score compared to those fed diets with glucogenic energy source.

Key Words: Glucogenic Diet, Goat Performance, Lactation, Lipogenic Diet

INTRODUCTION

Goat production in Indonesia is dominated by the smallholders based on the mixed-farming system or extensively grazing system. A more intensively and commercial goat production system where total-mixed ration (TMR) is practiced has been emerged, particularly in the densely populated area where land is becoming limited for agricultural activities while the demand for goat meat is increasing. TMR is considered to be suitable in providing nutrients in more precise amounts and balances as compared to conventional practices (Schingoethe 2017). This is particularly relevant during the lactation period when the nutrient and energy requirements are at the peak levels to support a high level of milk production (Goetsch 2019) and a reduction in feed intake may occur due to the metabolic and hormonal factors (van Knegsel et al. 2007). During the lactation state, the animals are therefore commonly experiencing a negative energy balance that will influence negatively their subsequent performances (van Knegsel et al. 2007). A feeding system that provides the animal's access to a high nutrient and energy density diet is thus required in order to prevent this phenomenon. Non-structural carbohydrate (NSC) or fat could be added to diet in order to increase the dietary energy density and total energy intake. However, the high concentration of both NSC and fat in the diet may provide negative effects on the ruminants. High NSC diet may increase the risk of a suboptimal rumen environment and could cause metabolic disorder such as acidosis (Wang et al. 2016; Shen et al. 2019), while high-fat diet may result in a decrease in fiber digestibility and forage intake (Weld & Armentano 2017). Cassava meal was used as the main glycogenic energy source because it contained relatively high non fiber carbohydrate. In addition, cassava meal is widely available with competitive cost. Bergafat was used as the main lipogenic energy source since it is a rumen-protected fat so that minimum rumen fermentation disorder could be prevented when providing at relatively high level in diet. The present study was aimed to investigate the responses of lactating goats in terms of feed and nutrient and energy intakes, body weight changes, body condition scores and blood metabolites on diets differing in the energy sources provided in the total-mixed ration.

MATERIALS AND METHODS

Animal and diets

The experiment was conducted at the Indonesian Goat Research Station using a total of 35 does having 2-3 parities and average body weight of 30.3±4.48 kg and BCS of 2.5±0.05 on a scale basis of 1 to 5. All animals were in the state of late pregnancy and each animal was in an individual crate 2 to 3 weeks prior to the expected kidding time. During this time all animals were offered the same total-mixed ration to adapt them to this type of feed presentation prior to the next lactation period. Animals were then divided into five groups and randomly allocated to one of the five lactation diet treatments for the whole lactation period. Diets are designed to be iso-nitrogenous and iso-caloric, but differing in the energy sources mainly by the differentiation of the inclusion rate of cassava meal as glucogenic energy source and fat as lipogenic energy source (Table 1). Bergafat (Berg+Scmidt) was used as a supplement to create lipogenic diet. This fat is a vegetable fat processed from palm oil and protected from rumen fermentation. Diet treatments were formulated such that the energy sources were mainly as glucogenic (TMR1), lipogenic (TMR5) or the combinations of glucogenic and lipogenic (TMR2, TMR3 and TMR4).

The forage to concentrate ratio of the experimental diets ranged from 25:75 to 35:65 (dry matter) using *I. zollingeriana* as the sole forage. The leaves of this leguminous tree were harvested every day in the morning and sun-dried immediately for 2 to 3 days before milled. All feed ingredients were mixed thoroughly using a horizontal mixer and were pelletized to yield total-mixed rations.

Experimental procedures

During the feeding trial lasting for 90 days, all does were fed *ad libitum* by allowing a refusal rate of 5 to 10 % twice daily in the morning (08:00) and in the afternoon (15:00). Each doe was confined in an individual cage of 1.5 x 2.0 m equipped with feed bunk designed to prevent the kid from access to the diets. Water was available all the time in a plastic container located outside the cage. Feed offered and refusals were recorded daily to calculate feed intake. Does and kids were weighed weekly and the does were scored for body conditions on the same date. Body condition scores were determined by a panel of three individuals as described by Ngwa et al. (2007). Samples of diets (0.5 kg) were taken weekly and put in the freezer (-20^0C) before analyses. The samples were composited and mixed thoroughly and subsampled for subsequent chemical analyses. Following the feeding trial, the animals were transferred into individual metabolism cage with a slatted wood floor for seven days as adaptation period and five days as the collection period. The animals were fed at a level allowing minimal feed residues. During the collection period diets were

Table1. Ingredient compositions of the experimental total mixed rations (TMRs)

Ingrediets, % DM	Diet				
	TMR1	TMR2	TMR3	TMR4	TMR5
I.zollingeriana leaf meal	25.0	25.0	30.0	30.0	35.0
Cassava meal	37.0	33.0	27.0	22.0	5.0
Soybean meal	20.0	20.0	17.0	16.0	10.0
Palm kernel meal	2.0	1.0	6.0	12.0	16.0
Bergafat	1.0	1.0	2.5	5.0	5.0
Rice bran	10.0	15.0	12.5	10.0	24.0
Bone meal	1.0	1.0	1.0	1.0	1.0
Limestone	1.0	1.0	1.0	1.0	1.0
Salt	1.0	1.0	1.0	1.0	1.0
Molasses	1.0	1.0	1.0	1.0	1.0
Mineral premix[a]	1.0	1.0	1.0	1.0	1.0

[a]Ultramineral: $CaCO_3$ (50%), P (25%), Mn (0.35%), I (0.10%), Cu (0.15%), Fe (0.8%), Zn (0.2%), Mg (0.15%), NaCl (23.05%)

sampled daily in polythene bags and total feces were weighed and samples (10% w/w) were taken and put in polythene bags. Feed and fecal samples were frozen at -20^{0}C for each animal for further chemical analysis.

The dry matter content of diets and feces was determined by drying the samples in the oven at 60^{0}C for 48 h and were ground to pass a 1-mm screen in a Willey mill. Crude protein and fat contents were determined by the procedures of AOAC (2007). Neutral Detergent Fiber was analyzed according to the procedures of Van Soest et al. (1991). The gross energy of the diets was determined by the adiabatic calorimeter. Blood was collected from all does at 4, 8 and 12 weeks postpartum at 3 hours after morning feeding. Blood (5.0 ml) was withdrawn from the jugular vein into vacutainer tubes containing lithium heparin. The tubes containing blood were centrifuged at 3,000 x g for 15 min, and plasma was separated and kept in freezer at -20^{0}C for subsequent analyses. The colorimetric assay was performed to measure the plasma glucose.

Statistical analysis

The experiment was conducted in a completely randomized design with five treatments and seven replicates. Data were analyzed using analysis of variance using the procedure of the General Linear Model of Statistical Analysis System (SAS 2012). Duncan's multiple range tests was used to compare differences among treatment means using 0.05 level of significance (Steel & Torrie 1980).

RESULTS AND DISCUSSION

The experimental diets were considered to be iso-nitrogenous with CP contents ranging from 18.2 to 18.8% and iso-caloric with the ME contents ranging from 2940 to 2976 Kcal/Kg DM (Table 2). The TMR1 is considered as a glucogenic diet as it contained the highest level of non-fiber carbohydrate and the lowest level of ether extract, while the TMR5 is considered as a lipogenic diet as its ether extract and non-fiber concentration are the highest and the lowest, respectively. The level of NDF in the diets ranged from 31 to 37%, which were in the safe level to prevent metabolic problems when feed is presented as a total mixed ration.

The level of DM intake ranged from 3.01 – 3.33 % BW in all diet treatments which is considered to be normal for lactating goats. This also indicates that providing diet in the form of pelleted totally mixed ration could be practiced in an intensive goat production system. There were no DM intake differences ($P>0.05$) as the fat content increased from 1.49% (TMR1) to 2.28% (TMR2), but significant increases ($P<0.05$) in DM intake were observed in goats fed higher fat and lower NFE diets (TMR3, TMR4 and TMR5). Goats have been suggested to be less susceptible to negative effects of high dietary fat level on dry matter intake (Goetsch 2019). In the present study, diets were isocaloric and were provided as total mixed rations in pellet form which could have negated the possible negative effect of fat on palatability. Using bergafat as lipogenic supplement which is typically protected from

Table 2. Chemical compositions of the experimental total mixed rations (TMRs)

Nutrien	Diet				
	TMR1	TMR2	TMR3	TMR4	TMR5
Dry matter (DM),%	89.76	90.08	89.93	90.12	89.84
Organic matter,% DM	90.14	90.12	90.36	90.18	90.49
Crude protein,% DM	18.64	18.87	18.19	18.78	18.69
Ether extract, % DM	1.49	2.28	4.74	6.08	7.46
Crude fibre,% DM	8.04	8.66	9.73	10.40	12.8
NDF,%DM	31.09	32.65	34.62	36.14	37.83
Non fibre carbohydrate[a],% DM	38.92	36.32	32.80	29.18	26.51
Gross energy, Kcal/kg DM	4742	4801	4709	4758	4767
Metabolizable energy[b],Kcal/kg DM	2940	2976	2920	2950	2956

Results analysis of laboratory (AOAC 2007)
[a]Calculated according to Van Soest et al. (1991); [b]estimated using NRC (1981)

Table 3. Nutrient intake (g/head/d) and metabolizable energy intake (ME, Kcal/head/d) and nutrient digestibility (%) in lactating goat fed diets of different energy sources

Parameters	Diet				
	TMR1	TMR2	TMR3	TMR4	TMR5
Intake					
Dry matter	889±29.4[a]	926±31.6[a]	987±36.9[b]	981±27.5[b]	1029±32,5[b]
Organic matter	827±25.3[a]	844±27.6[a]	905±31.8[b]	902±26.7[b]	940±28.4[b]
Crude protein	166±7.1[a]	175±8.2[a]	180±9.1[a]	184±9.7[b]	192±9.4[b]
Ether extract	13.4±0.7[a]	21.2±1.1[b]	46.9±2.3[c]	67.3±2.8[d]	76.1±2.7[d]
ME, Kcal/day	2614±116[a]	2756±135[b]	2882±115[c]	2893±147[d]	3041±146[d]
NFC	346±17.9[a]	336±15.1[a]	323±16.8[a]	286±20.1[b]	273±14.2[b]
NDF	277±13.3[a]	303±14.8[a]	341±14.5[b]	354±13.3[b]	388±12.5[c]
Digestibility					
Dry matter	63.9±1.6[a]	65.2±2.7[a]	69.1±2.6[b]	68.2± 2.5[b]	71.5±2,4[b]
Organic matter	65.4±1.7[a]	66.9±1.2[a]	70.8±2.1[b]	70.3±2.3[b]	73.6±1.4[b]
Crude protein	67.4±1.4[a]	68.7±1.9[a]	72.2±1.5b[ab]	72.5±1.4[ab]	76.2±1.5b[b]
Ether extract	73.8±2.1[a]	73.5±1.1[a]	78.3±2,3[a]	85.2±2.3[b]	87.8±2.0[b]
NFC	91.5±3.8	92.2±4.3	89.7±3.1	90.4±2.6	91.1±3.7
NDF	58.4±2,3[a]	59.2±1.6[a]	54.8±1.7[b]	52.4±2.5[ab]	51.3±1.3[b]

[a,b,c,d] Different superscripts in the same row significantly differ (P<0,05)

the rumen fermentation could have been also an important factor. The lower feed intake in glucogenic diet (TMR1 and TMR2) could also be related to the high NFC content. Although the experimental diets contained 25% roughage the decrease of rumen pH in the glucogenic diets was expected since the glucogenic contain a high level of cassava meal (33-37%), which is rapidly degraded in the rumen. This low pH may result in less feed intake. Although the rumen pH was not measured in the present experiment, Shen et al. (2019) observed that the rumen pH decreased to 5.36 when the high starch diet was fed to a goat. In the present study, the starch is represented by NFC and ranged from 36-39%. Mudgal et al. (2012) however did not find a significant effect of supplementing bypass fat on feed intake in lactating crossbred cows.

There was a consistent increase in intake and digestibility of DM, OM, CP and EE as the fat content of diet increased, but NDF digestibility decreased as the fat content of diet increase. This is consistent with Atikah et al. (2018) that CP and EE digestibility in goat increased with the increasing level of fat in the diet. Bhatt et al. (2013a) however indicated that the digestibility of NDF, EE and CP was improved when Ca protected fat was supplemented in ewes. The increased CP intake is associated with greater DM intake. The greater CP intake and digestibility resulted in greater intake of digestible protein as the fat content of the diet increased. The digestible protein intake ranged from 111 to 146 g/d in all treatments which is predicted to be sufficient for lactating goats.

Bodyweight changes of the lactating goats are presented in Table 4. All animals gained during the lactation period with ADG ranged from 15 to 46 g indicating positive energy balances. Goat receiving more glucogenic diets (TMR1 and TMR2) gained the least and those receiving the most lipogenic diet (TMR5) gained most ($P<0.05$). The ADG linearly increased as the percentage of fat in diets increased up to 7.5% i.e. as the diet becoming more lipogenic. This is consistent with Vakili et al. (2011) indicating that diet with the fat content of 6.7% had potential to improve the energy balance state of sheep compared to a glucogenic diet. Consistent findings were also reported by Bhatt et al. (2013b) in lambs and Bhatt & Sahoo (2017) in ewes that growth was improved in diet with higher fat content. In tropical dairy cows (Mobeen et al. 2017) and in non-lactation ewes, however, Behan et al. (2019) did not found a significant effect of increasing the fat content of diet from 4.9 to 8.4% by supplementing protected fat. In the present study, body condition scores were also improved in lactating goat received more lipogenic diets. These increased in body weight and body condition scores might have related to greater DM and nutrient intakes and efficiency of energy utilization (Park et al. 2010; Bhatt & Sahoo 2017). The ration of high producing animal like a lactating goat should contain 4 to 6% fat of natural and protected fat (Naik 2013), while in dairy cow, Palmquist & Jenkins (2017) suggested that more than 3% fat in diet is required to maintain appropriate body condition. The efficiency of feed utilization as indicated by the FCR value (g DM intake/g ADG) showed that lactating goats utilized feed more efficiently for body weight gain as the energy source of the diet was more lipogenic ($P<0.05$). In the present study, the FCR for body weight gain ranged from 22.15 (lipogenic diet) to 58.79 (glucogenic diet). This low efficiency of feed utilization for body weight gain was indicative of more diet that has been directed for milk production.

Blood glucose concentration (Table 5) was not affected by diet treatments nor by lactation period ($P>0.05$). The blood glucose levels in all diet treatments ranged from 53 to 58 mg/dl. Numerically, the blood glucose level tends to increase as the diet becomes more lipopgenic. The blood glucose concentrations were consistent with feed intake for all treatments. These levels are consistent with Singh & Ludri (2002) reporting the blood glucose level in 30, 60 and 90 lactation periods between 49-57 mg/dl. Range of glucose levels of 60 to 71 mg/dl in several breeds of the goat during lacatatin maintained under intensive production system was reported by Mohammed et al. (2016). It was expected the blood glucose level in goats offered glukogenic diet was higher than the lipogenic group since more propionic acid would have been produced from NFC fermentation in the rumen which is an important glucose precursor through the gluconeogenesis pathway. The non-significant effect ($P>0.05$) observed in the present study could be due to similar gluconeogenesis rate using amino acids as precursor of glucose in the lipogenic group. During the lactation period the blood insulin level is commonly decreasing in ruminant (Karapehlivan et al. 2007), and this may relate to the relatively high blood glucose level in the lactating goat as observed in the current experiment. The lower level of blood glucose in non-lactating goats of 46 – 48 mg/dl has been reported (Bansode et al. 2019).

The blood urea concentration (Table 5) was not affected by diet treatment nor by the lactation period ($P>0.05$). The blood urea concentration in all diet treatments ranged from 37 - 43 mg/dl. Numerically, the blood urea levels were higher in the mid and late lactation as compared to early lactation. The lower blood urea concentration in the early lactation is expected since it is related to the level of protein intake and feed intake which is usually lower during the period. Kohn et al. (2005) in his review indicated that the range of blood urea concentration between 25 and

Table 4. Body weight changes and body condition scores of lactating goat offered diets of different energy sources

Parameter	Diet				
	TMR1	TMR2	TMR3	TMR4	TMR5
Initial BW,kg	29.13±1.33	29.15±1.25	28.28±1.26	27.36±1.47	28.76±1.59
Final BW,kg	30.50±1.91	30.40±1.59	30.92±1.06	30.32±1.15	32.94±1.66
BW change,kg	1.4±0.07[a]	1.3±0.08[a]	2.6±0.11[b]	3.01±0.15[c]	4.17±0.11[d]
ADG,g	15.12±0.98[a]	14.55±1.43[a]	28.32±2.97[b]	33.89±1.24[c]	46.45±2.11[d]
FCR	58.79±3.53[a]	63.6±4.45[a]	34.87±1.67[b]	28.9±1.88[c]	22.15±1.55[d]
BCS	2.56±0.25[a]	2.53±0.19[a]	2.69±0.23[b]	2.76±0.21[b]	2.73±0.19[b]

[a,b,c,d] Different superscripts in the same row significantly differ (P<0,05); FCR:DM intake/ADG (g/g)

Table 5. Blood glucose and urea-N concentration in lactating Boerka goat offered total mixed ration differing in energy sources

Lactation, days	Diet				
	TMR	TMR2	TMR3	TMR4	TMR5
			Blood glucose, mg/dl		
30	53,01± 4,82	54,65± 3,97	58,85± 4,85	57,80± 2,66	58,18± 11,9
60	54,17±4,03	52,67± 3,89	53,65± 4,45	56,74± 5,13	55,50± 3,18
90	54,51± 4,85	54,14± 3,81	58,37± 3,96	58,44± 2,95	57,49± 4,92
			Blood urea-N,mg/dl		
30	38,58±2,49	34,48±2,11	37,65±2,23	35,60±2,93	37,50±2,51
60	41,14±2,74	39,0± 3,20	41,64±1,27	42,20±3,31	39,19±3,17
90	41,35±1,19	41,40±1,59	42,28±2,86	43,22±2,16	42,75±3,51

38 mg/dl. Maintained under intensive production system Mohammed et al. (2016) observed urea blood levels of 27 to 37 mg/dl in different breeds of goat. The relatively high blood urea level observed in the present experiment may be related to the relatively high intake of crude protein (166 - 192 g/d) from diet with 18% crude protein level. In addition, the major protein source of all diet treatment was leguminous leaves of *I.zollingeriana* of high rumen degradability (Tarigan et al., 2010) and soybean meal which is highly soluble in the rumen. The non-significant effect (P>0.05) of diets differing in crude protein intake with similar protein degradation rate on the level of urea blood concentrations indicated that there was sufficient energy for microbial utilization of nitrogen in the rumen for optimum microbial protein synthesis in all diet treatments. The relatively high blood urea levels observed in the present experiment, however, are comparable to 40 – 56 mg/dl observed by Adiwinarti et al. (2018) in Kacang goat fed complete feed. Kumagai & Ngampongsai (2006) indicated a linear relationship between an increased blood urea levels with the crude protein level of diet in goats. Thus, it is indicated from this experiment that lipogenic or glucogenic diets did not affect the concentration of urea blood level.

CONCLUSION

Presentation of feed as pelleted total mixed-ration provides normal feed and nutrient intakes in goat during the whole lactation period. Compared to lactating goats fed diet with higher glucogenic energy, those offered diet with more lipogenic energy by increasing the fat content of the diet up to 7.5% responded better in term of feed intake, nutrient intakes, ADG and BCS. As the protected fat is used to increase the energy content of the lipogenic diets, it is uncertain, however, wether these responses have been consistent when the energy lipogenicity of the diet has been increased by increasing the fat content level greater than 7.5% or when non protected fat has been used.

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The Effect of *Morinda citrifolia* and *Arthrospira plattensis* Powder on the Performance and Quality of Broiler Duck Carcasses

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ABSTRAK

Kurniawan D**,** Christie CDY**.** 2020. Pengaruh pemberian tepung *Morinda citrifolia* dan *Arthrospira plattensis* terhadap kinerja dan kualitas karkas itik pedaging. JITV 25(1): 34-38. DOI: http://dx.doi.org/10.14334/jitv.v25i1.2053.

Penelitian ini bertujuan mengetahui kinerja dan kualitas karkas itik pedaging yang diberi tepung *Morinda citrifolia* dan *Arthospira platensis*. Sebanyak 168 ekor itik pedaging (*unsexed*) yang berumur 2 minggu dengan rata-rata bobot badan 463±29,38 g dan tingkat keragaman 6,35 %, dikelompokkan ke dalam 28 petak masing-masing berisi 6 ekor. Metode penelitian yang digunakan adalah uji biologis yang dirancang dengan rancangan acak lengkap (RAL) yang terdiri dari 7 perlakuan dan 4 ulangan yaitu T0 (pakan basal sebagai kontrol), T1 (pakan basal + 0,2% bubuk *Morinda citrifolia* (MP)), T2 (pakan basal + 0,5% bubuk *Arthrospira plattensis* (AP)) , T3 (pakan basal + 0,2% of MP+ 0,5% of AP), T4 (pakan basal + 0,4% of MP + 0,5% of AP), T5 (pakan basal + 0,2% of MP + 0,1% of AP), T6 (pakan basal 0,4% of MP + 0,1% of AP). Parameter yang diuji adalah konsumsi pakan, pertambahan bobot badan, konversi pakan, persentase karkas, lemak abdominal dan organ dalam. Data yang diperoleh dianalisa menggunakan analisa ragam ANOVA, apabila terdapat perbedaan dilanjutkan dengan uji jarak berganda Duncan's. Hasil penelitian menunjukkan bahwa perlakuan tidak berpengaruh nyata (P>0,05) terhadap konsumsi pakan, pertambahan bobot badan dan konversi pakan. Perlakuan juga tidak berpengaruh nyata (P>0,05) terhadap persentase karkas, lemak abdominal dan organ dalam. Pemberian pakan dengan tepung *Morinda citrifoli*a dan *Arthospira platensis* tidak dapat meningkatkan produktifitas dan kualitas karkas itik pedaging.

Kata Kunci: Itik Pedaging, Kinerja, Kualitas Karkas

ABSTRACT

Kurniawan D**,** Christie CDY. 2020. The effect of *Morinda citrifolia* and *Arthrospira plattensis* Powder on Performance and Quality of Broiler Duck Carcasses. JITV 25(1): 34-38. DOI: http://dx.doi.org/10.14334/jitv.v25i1.2053.

This study was aimed to investigate performance and quality of broiler duck carcasses fed with *Morinda citrifolia* and *Arthrospira plattensis*. A total of 168 two-week-old broiler duck with an initial average body weight of 463 ± 29.38 g and a diversity of 6.35 % were randomly allotted to 7 experimental groups with 4 replications each with 6 bird per replication. Treatments were T0 (basal diet as a control), T1 (basal diet + 0.2% of *Morinda citrifolia* powder (MP)), T2 (basal diet + 0.5% of *Arthrospira plattensis* powder (AP)) , T3 (basal diet + 0.2% of MP+ 0.5% of AP), T4 (basal diet + 0.4% of MP + 0.5% of AP), T5 (basal diet + 0.2% of MP + 0.1% of AP), T6 (basal diet 0.4% of MP + 0.1% of AP). Variables measured were feed intake, body weight gain, feed conversion ratio, carcass percentage, abdominal fat, and visceral organ. Data were analyzed for variance based on a Completely Randomize Design and continued with Duncan's multiple Range Test for differences. Result showed that the treatments did not affect (P>0.05) on feed intake, body weight gain and feed conversion ratio. The treatments also did not affect (P>0.05) carcass percentage, abdominal fat, and visceral organ. The diet did not significantly improve performance and quality of broiler duck carcasses.

Key Words: Broiler Ducks, Performance, Carcass Quality

INTRODUCTION

Some plants that are rich in bioactive compounds and have the potential as phytobiotics in livestock such as *Morinda citrifolia* and *Arthrospira platensis*. *Morinda citrifolia* contains nutritional values such as minerals, vitamin, carbohydrates, and other nutrients which directly or indirectly help in metabolizing nutrients and good for cell and tissue growth (Abou Assi et al. 2017). Broilers fed fresh *Morinda citrifolia* juice (1.5 ml/head/day) showed better production performance on body weight gain, feed conversion and

feed efficiency (Sunder et al 2011). The use of 5% *Morinda citrifolia* extract in feed showed better body weight gain, growth, and performance of egg production in quails (Sunder et al. 2015).

Arthrospira platensis contain 60-70% digestible protein with all essential amino acids, unsaturated fatty acids such as ɣ-linolenic acid, vitamins especially vitamin B12 and provitamin A and mineral especially iron and various photosynthetic pigments (Hosseini et al. 2013). The bioactive components of *Arthrospira platensis* include phycocyanin, β-carotene, ɣ-linolenic acid and phenolic compounds that make *Arthrospira platensis* has antioxidant, antimicrobial and immune-stimulant properties, thus avoiding various diseases (Sudha et al 2011). Gružauskas et al. (2004) reported that *Arthrospira platensis* improved absorption of minerals and optimize nutrient digestion processes. Feeding *Arthrospira platensis* containing diets may increase the lactobacillus population and enhance the absorbability of dietary vitamins (Mariey et al. 2012). Antimicrobial activity in *Morinda citrifolia* as a feed additive is expected to improve the condition of microflora in the digestive system of poultry, especially in the small intestine. Good microflora conditions in the small intestine improve the absorption of feed substances so that feed digestibility and production performance improved. The antioxidant activity of *Arthrospira platensis* is expected to reduce or prevent damage by the oxidation process in quality poultry products. It is necessary to study effect of *Morinda citrifolia* and *Arthrospira platensis* in feed on productivity of broiler ducks which includes feed consumption, body weight gain, feed conversion and carcass quality like carcass percentage, abdominal fat, and internal organ weight.

MATERIALS AND METHODS

Birds and dietary treatments

One hundred sixty-eight hybrid broiler ducks with an initial body weight of 463±29.38 g were used in this study. The ducks were obtained from a local breeder. Completely randomized design with 7 treatments and 4 replications, 6 ducks per replication was arranged for this study. Treatments were T0 = basal diet, T1 = T0 + 0.2% *Morinda citrifolia* powder (MP), T2 = T0 + 0.5% Arthrospira platensis powder (AP), T3 = T0 + 0.2% MP + 0.5% AP, T4 = T0 + 0.4% MP+ 0.5% AP, T5 = T0 + 0.2% MP + 1% AP, T6 = T0 + 0.4% MP + 1% AP. The *Morinda citrifolia* and *Arthrospira platensis* powder addition were in the percentage of total diet basis. Composition and nutritional content of the basal diet are presented in Table 1.

Experimental bird management

This research was started with the preparation of *Morinda citrifolia* powder, *Arthrospira platensis* powder, diet, cage, and its equipment. *Morinda citrifolia* powder was obtained from Materia Medica (Batu, East Java, Indonesia). *Arthrospira platensis* was obtained from PT. Neoalga Indonesia Makmur (Sukoharjo, Central Java, Indonesia). The hybrid ducks from a local breeder were offered dietary treatments from one day to 42 days old. The birds from 1 to 14 days old were kept in litter cage, and they were moved and kept in the colony cages. Feeds and water were provided ad libitum throughout the study period. The experiment was conducted according to the standard procedures of rearing and treating farm animals as stated in the law of the Republic of Indonesia, number 18, 2009, concerning animal husbandry and health. Bodyweight and feed intake were measured weekly. The feed conversion ratio was determined as the feed intake per weight gain. At day 42, a total of 28 ducks were slaughtered, de-feathered, and eviscerated. The internal organs were immediately taken out and weighed. The following parameters were evaluated (in %, in relation to live body weight and weight of eviscerated carcass without neck): eviscerated carcass without neck, head, and neck, quarter anterior of carcass, quarter posterior of carcass, wings with skin, breast and leg with skin, abdominal fat and visceral organ (heart, liver, gizzard, spleen, and giblet).

Statistical analysis

Data were analyzed based on a Completely Randomized Design by ANOVA. Significant differences among treatment groups were further analyzed using Duncan's multiple-range test. A significant level of $p<0.05$ was implemented.

RESULTS AND DISCUSSION

Performance

Effect of dietary *Morinda citrifolia* and its combination with *Arthrospira platensis* powder on performance of broiler ducks are presented in Table 2. The result showed that the treatments did not affect ($P>0.05$) feed intake. This may be due to iso-calorie and iso-nitrogenous dietary treatments used in this study which led to the same feed intake. Several previous studies also noted that *Morinda citrifolia* powder and *Arthrospira platensis* powder had no effect on feed intake in broiler chicken (Abd El-Hady & El-Ghalid. 2018); (Mirzaie et al. 2018).

Table 1. The composition and nutritional content of basal diet

Ingredients	Amount
Maize, (% as fed)	56.15
Soybean meal, (% as fed)	22.80
Meat Bone Meal, (% as fed)	7.20
Polished rice, (% as fed)	7.10
Lime Stone, (% as fed)	3.80
Palm Oil, (% as fed)	2.00
Premix, (% as fed)	0.50
Dicalcium Phosphate, (% as fed)	0.27
Salt, (% as fed)	0.18
Analyzed composition, % of DM#1:	
Gross Energy, (cal/g)	3428
Crude Protein, (%)	18.69
Crude Fat, (%)	2.53
Fiber, (%)	1.31
Ash, (%)	11.11
Calcium, (%)	4.73
Phosphorus, (%)	0.76

#1 Analyzed by Laboratory of Animal Nutrition and Feed Science, Blitar Distric's Livestock and Fisheries Service

A number of studies have revealed the consistent benefits of *Arthrospira platensis* on the growth performance of broiler chickens. Kaoud (2012) and (Jamil et al. 2015) reported that the addition of *Arthrospira platensis* powder to the diet improves weight gain and decreased feed conversion of broiler chickens. Based on these published data the author inferred the dietary *Arthospira platensis* on broiler ducks. The present results showed that the treatments did not give significant (P>0.05) effect on body weight, weight gain, and feed conversion ratio. This finding was similar to that of Mirzaie et al. (2018), which reported that supplementation of 1% *Arthrospira platensis* did not affect performance characteristics in broilers chickens. Sugiharto et al., (2018) reported that the period during which *S. platensis* was supplemented in broiler feed did not affect the growth performance of broilers. Irrespective of feeding duration, dietary supplementation with 1% of *S. platensis* resulted in a corresponding effect on growth performance when compared to feeding zinc bacitracin as a growth promoter to broiler. Abd El-Hady & El-Ghalid (2018) reported that *S. platensis* supplementation to broiler diet improved feed conversion ratio. The improvement of FCR as a result of *S. platensis* supplementation could be attributed to the increase in body weight accompanied with no effect in feed intake.

Carcass quality

Data on comparison of the treatments of dietary *Morinda citrifolia* and *Arthrospira platensis* powder on quality of boiler duck carcasses are shown in Table 3. There were no significant (p >0.05) different values of variables affected by treatments. This is in agreement with previous findings (Sugiharto et al. 2018) which also reported that there was no effect of dietary *Arthrospira platensis* on carcass traits of broilers chicks observed for 35 days. Several studies reported that feeding *A. platensis* increased carcass percentage of broiler chicks (Koud, 2012, Mariey et al. 2014) and Japanese quail (Jamil et al., 2015). The precise reason for these different results is not known, but the relatively similar final bodyweight may result in a similar carcass percentage of broiler among the treatment groups in the present study. This inference was supported by Mariey, et al. (2014) who suggested that carcass weight was in parallel with the live body weight of broilers. That is, the increased carcass weight

Table 2. Effect of feeding *Morinda citrifolia* and *Arthrospira platensis* powder on performance production of broiler ducks

Variables	Treatments							SEM	*p*
	T0	T1	T2	T3	T4	T5	T6		
Feed intake (g/bird)	3231	3071	3221	3148	3127	3223	2947	41.00	0.52
Live body weight (g/bird)	1429	1346	1315	1404	1390	1470	1397	21.95	0.62
Weight gain (g/bird)	982	899	854	920	936	984	936	19.30	0.60
Feed Conversion Ratio	3.29	3.42	3.77	3.42	3.34	3.27	3.15	0.07	0.38

[1)] T0 = basal diet, T1 = T0 + 0.2% *Morinda citrifolia* powder (MP), T2 = T0 + 0.5% *Arthrospira platensis* powder (AP), T3 = T0 + 0.2% MP + 0.5% AP, T4 = T0 + 0.4% MP+ 0.5% AP, T5 = T0 + 0.2% MP + 1% AP, T6 = T0 + 0.4% MP + 1% AP

Table 3. Effect of feeding *Morinda citrifolia* and *Arthrospira platensis* powder on quality of broiler duck carcasses.

Variable	Treatments							SEM	*p*
	T0	T1	T2	T3	T4	T5	T6		
Eviscerated carcass (g)	1028	860	893	1001	919	1115	948	24.94	0.07
Dressing (%)	60.51	59.11	58.96	60.57	57.37	62.13	60.32	0.70	0.72
As % of dressed carcass									
Quarter anterior (%)	27.74	26.69	27.23	27.20	27.39	27.35	27.47	0.22	0.17
Quarter posterior (%)	21.94	22.75	22.36	22.74	22.16	22.16	22.13	0.20	0.20
Breast (%)	12.00	10.73	9.99	13.75	12.14	10.80	9.40	0.58	0.82
Thigh (%)	10.32	11.04	11.00	11.29	11.14	10.66	12.00	0.35	0.96
Wing (%)	7.66	7.82	7.34	6.43	6.92	6.89	7.31	0.15	0.14
Head and neck (%)	17.09	18.40	18.12	16.52	18.12	16.15	16.97	0.42	0.77

[1)] T0 = basal diet, T1 = T0 + 0.2% *Morinda citrifolia* powder (MP), T2 = T0 + 0.5% *Arthrospira platensis* powder (AP), T3 = T0 + 0.2% MP + 0.5% AP, T4 = T0 + 0.4% MP+ 0.5% AP, T5 = T0 + 0.2% MP + 1% AP, T6 = T0 + 0.4% MP + 1% AP

Table 4. Effect of feeding *Morinda citrifolia* and *Arthrospira platensis* powder on visceral organ of broiler ducks

Item (% live BW)	Treatments							SEM	*p*
	T0	T1	T2	T3	T4	T5	T6		
Liver	2.99	2.59	3.02	2.85	3.46	2.45	2.56	0.13	0.32
Gizzard	3.14	3.70	3.07	3.21	3.24	3.07	3.12	0.08	0.52
Heart	0.47	0.55	0.57	0.50	0.51	0.54	0.60	0.01	0.62
Spleen	0.06	0.07	0.13	0.06	0.07	0.08	0.09	0.005	0.70
Giblet	6.60	6.84	6.66	6.56	7.22	6.06	6.27	0.14	0.84
Abdominal fat	1.36	0.70	0.95	1.73	1.15	1.24	0.90	0.12	0.26

[1)] T0 = basal diet, T1 = T0 + 0.2% *Morinda citrifolia* powder (MP), T2 = T0 + 0.5% *Arthrospira platensis* powder (AP), T3 = T0 + 0.2% MP + 0.5% AP, T4 = T0 + 0.4% MP+ 0.5% AP, T5 = T0 + 0.2% MP + 1% AP, T6 = T0 + 0.4% MP + 1% AP

and total edible part were attributed to the final live bodyweight of broilers and vice versa

Visceral organs

Data describing the effect of dietary *Morinda citrifolia* and *Arthrospira platensis* powder on visceral organs of broiler ducks are summarized in Table 4. No significant different effects ($p>0.05$) were found among treatments on liver, gizzard, heart, spleen, giblet and abdominal fat of broiler ducks. Nurhayati (2010) reported that using *Morinda citrifolia* powder in the ratio up to 10 percent did not significantly affect internal organ of broiler chickens. This is in agreement

with (Sugiharto et al. 2018) that feeding of *Arthrospira platensis* (1%) had no significant effect on internal organ of broiler chicks for 35 days. Total organ weight was found to be lower in bird fed with antibiotics as well as 0.05% *Moringa* fruit powder. A significant effect of herbal dietary treatment was not observed on subcutaneous fat content (neck, breast, and leg) of broiler carcass and on abdominal fat content (heart and vent) except the fat around gizzard (David et al. 2012).

CONCLUSION

The finding of the present study suggests that the dietary of *Morinda citrifolia* and *Arthrospira platensis* powder did not significantly improve performance and quality of broiler ducks carcasses.

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