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Original study

Effect of feed form, pellet diameter and enzymes supplementation on carcass characteristics, meat quality, blood plasma constituents and stress indicators of broilers

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Abstract

Four hundred-twenty broilers were used in a factorial design (4×3) in which four feed forms (mash vs. pellet diet with diameter of 2 mm, 3 mm and 3.5 mm, respectively) and three enzymes treatments (unsupplemented, phytase, phytase+multi-enzyme) were used. Each treatment was replicated five times (7 broiler/replicate). Feed form had no effects on most of carcass traits and physical characteristics of meat, but pellet diets decreased the relative weight of gizzard and caecum length. Feeding 3.5 mm pellet diets increased abdominal fat compared to that of broilers fed mash diets. Pellet with 3 mm diameter increased and decreased respectively meat fat and moisture. Pellets with 3.5 mm diameter increased meat ash compared to broilers fed mash diet. Levels of plasma glucose and alanine aminotransferase of broilers fed 2 mm pellet, cholesterol of broilers fed 3 mm pellet and albumin/globulin ratio, monocytes and red blood cells of broilers fed 3.5 mm pellet were significantly higher than those of broilers fed mash diet. Haemoglobin, phagocytic activity, heterophils and heterophils/lymphocyte ratio were higher in groups fed mash diets and 2 mm pellet. Packed cell volume and phagocytic index were the highest in group fed mash diets. Phytase or multi-enzyme+phytase increased carcass yield, total edible parts and decreased inedible parts compared to broilers fed diet without enzyme supplementation. Enzyme supplementations significantly and similarly increased plasma glucose, total protein, triglycerides, red blood cells and phagocytic activity and decreased phagocytic index in comparison to the control

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group. However, phytase alone decreased cholesterol and increased monocytes by contrast with the control group. Multi-enzymes+phytase induced greater effect on white blood cells than phytase alone.

Keywords: broilers, feed form, multi-enzymes, phytase, blood parameters, carcass characteristics, meat quality

Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; H/L ratio: heterophils/lymphocyte ratio; PA: phagocytic activity; PCV: packed cell volume; PI: phagocytic index; RBC: red blood cells; WBC: white blood cells

Introduction

The physical forms of diets and feed particle size have a great effect on poultry yield (Amerah *et al.* 2007). Agunbiade (2000) and Adeyemi *et al.* (2008) reported that higher percentage of dressing, breast meat, drumstick and thigh, which are the most expensive commercial cuts of the chicken, were obtained in birds fed pelleted diets. Hassan & El-Sheikh (2010) showed that both carcass and giblets percentages were not affected by feed form, while gizzard percentage and digestive tract weight decreased in groups fed pellets in comparison to mash diets. Also, Twina *et al.* (1994) found that the relative weight of the gizzard was reduced by pellet. Several authors also found an effect of feed form (mash or pellet) on some blood parameters as total protein, albumin, serum ALT and aspartate aminotransferase (AST) (Andi *et al.* 2011, Corzo *et al.* 2012).

The use of phytase or enzyme cocktails containing phytase can further improve broilers' performance. Ismail *et al.* (2006) found that enzyme cocktails significantly decreased intestinal percentage, while no significantly effects on carcass yields and internal organs percentage (proventriculus, gizzard, liver and heart) of broilers were observed. The relative weight of the breast, legs, liver, and gizzard as percentage of live weight were unaffected by enzyme supplementations (Shirzadi *et al.* 2009). Similar results were found for carcass yield, which was higher in enzyme supplemented broilers (Abudabos 2010, 2012). Kidd *et al.* (2001) and Attia *et al.* (2003a,b) found that multi-enzymes and phytase had no effects on carcass yields and internal organs of broilers. Biochemical constituents of blood plasma including plasma total protein with their fractions, plasma total lipids, cholesterol and liver enzymes (AST and ALT) were not affected by enzymes (Al-Harthi, 2006). Also, Qota *et al.* (2002) found that cell-wall degrading enzymes and/or phytase supplementation had no adverse effect on biochemical constituents of plasma and liver function of broilers. Abudabos (2012) reported that serum total protein, calcium and phosphorus were affected by enzymes.

The aim of this work was to investigate the effect of feed form (mash vs. pellet), pellet diameter (2, 3 and 3.5 mm) and enzyme supplementation (phytase vs. an enzyme cocktail containing xylanase, amylase, subtilisin and phytase) on carcass characteristics, meat quality, blood plasma constituents and stress indicators of broilers from 21 to 37 d of age.

Material and methods

Experimental design

A total of 420 twenty-one day old Arbor acres broilers were randomly distributed among 60 cages (seven birds per cage). The cages were equally divided into four groups (15 cages per group) and broilers were submitted to the following dietary treatments up to 37 d of age (grower period 21-29 d, finisher period 30-37 d). The groups fed, respectively: mash diet; pellet diet with 2 mm diameter in both grower and finisher periods; pellet diet with 2 mm diameter in grower and 3 mm diameter in finisher period; pellet diet with 3 mm diameter in grower and 3.5 mm diameter in finisher period. Each group was divided into three subgroups (five cages/subgroups) whose diets were unsupplemented (control subgroups) or supplemented with phytase, Phyzyme XP (0.07 g/kg diet) or multi-enzyme containing Avizyme 1505 (0.2 g/kg diet) plus Phyzyme XP (0.07 g/kg diet). Pelleting temperature did not exceed 80 °C. Phyzyme and Avizyme are products of Danisco Animal Nutrition (Marlborough, Wiltshire, UK). Avizyme 1505 is a multi-enzyme containing 1 500 U/g endo-1, 4-β-xylanase, 2 000 U/g α-amylase and 20 000 U/g subtilisin. Phyzyme XP is an Escherichia coli phytase classified as a 6-phytase with hydrolysis of the phosphate moiety being initiated at the 6-position on the phytate molecule. The feeding system and husbandry practice during 1-20 d of age are presented by Attia *et al.* (2012).

The experimental diets were formulated according to NRC (1994). Chemical analysis of diets was according to AOAC (2004). Ingredients and chemical composition of the diets (as fed basis) fed during the grower and finisher periods are shown in Table 1. The available P (avP) and Ca contents were adjusted in the diets supplemented with phytase according to phytase equivalent values (Attia 2003a, b, Attia *et al.* 2003a, Choct 2006).

Housing and husbandry

Broilers were housed in battery brooders in semi-opened house. Each cage (60 L×45 W×35 H) was equipped with 60 cm type feeders and one nipple drinker. Broilers were fed ad libitum the experimental diets and given free access to water. A light schedule was 20 h light during 21st to 34th d of age followed by 24 h of light until slaughter. The average outdoor minimum and maximum temperature and relative humidity during the experimental period were 21.2 and 24.2 °C and 56.7 and 58.7 %, respectively. The housing temperature was 24 °C at 21 d of age, declined gradually to 21 °C at 28 d of age and was then stabilized until slaughter. Chicks were vaccinated against most common diseases such as Newcastle disease, avian influenza, infectious bursa disease and infectious bronchitis.

Slaughter test

At 37 d of age, six broilers (three of each sex) were taken randomly from each treatment and slaughtered. The remaining carcass after bleeding, plucking and eviscerating was weighed (dressing percentage), divided into breast and hind parts and weighed. Liver, gizzard, heart and spleen were separated and individually weighed. The carcass parts were expressed as relative to live body weight. The edible parts included the empty carcass plus giblets (liver, gizzard and heart), while inedible parts included feather, blood, head, digestive tract and

spleen. A sample of breast meat and thigh meat (50:50 basis) was analysed for dry matter, protein, fat and ash according to AOAC (2004). Meat tenderness was measured according to the method of Volovinskaia and Kelman (1962). Colour intensity of meat was determined according to the method of Husani *et al.* (1950).

Table 1
Ingredients and chemical composition of the experimental diets

	Grower diets (21-29 d)			Finisher diets (30-37 d)		
	Without	Multi-Enzymes	Phytase	Without	Multi-Enzymes	Phytase
Ingredients, g/kg						
Maize	518.5	518.5	518.5	560.0	560.0	560.0
Soybean meal (44 %)	244.2	244.2	244.2	280.0	280.0	280.0
Full fat soybean meal	130.0	130.0	130.0	0.0	0.0	0.0
Rye	50.0	50.0	50.0	70.0	70.0	70.0
Vegetable oil blend	20.0	20.0	20.0	53.0	53.0	53.0
Dicalcium Phosphate	16.0	11.0	5.50	16.0	11.0	5.50
Limestone	10.0	10.0	10.0	10.0	10.0	10.0
NaCl	4.5	4.5	4.5	4.5	4.5	4.5
Vit+min premix[1]	3.0	3.0	3.0	3.0	3.0	3.0
DL-Methionine	2.0	2.0	2.0	2.0	2.0	2.0
L-Lysine	1.5	1.5	1.5	1.5	1.5	1.5
Washed building sand	0.30	5.03	10.73	0.0	4.73	10.43
Avizyme 1505	0.0	0.2	0.0	0.0	0.2	0.0
Phyzyme xp[2]	0.0	0.07	0.07	0.0	0.07	0.07
Determined* and calculated** chemical-nutritional composition, g/kg						
Dry matter*	875.5	875.0	876.1	870.0	870.3	870.6
ME, MJ/kg**	12.98	12.98	12.98	13.38	13.38	13.38
CP, %*	211.2	211.0	211.4	185.3	185.5	185.0
Crude fat, %*	64.5	64.3	64.7	68.0	68.3	67.8
Crude fibre, %*	35.1	35.3	34.9	38.0	38.2	38.3
Ash, %*	54.8	54.8	54.8	57.0	57.0	57.0
Lysine, %**	12.3	12.3	12.3	10.4	10.4	10.4
Methionine, %**	5.2	5.2	5.2	4.8	4.8	4.8
Meth+cystine**	8.7	8.7	8.7	7.8	7.8	7.8
Calcium**	8.5	7.4	6.4	8.3	7.2	6.2
Total P**	5.8	4.9	3.9	5.3	5.3	4.3
Average P**	4.1	3.2	3.2	4.1	3.2	3.2
NFE, %**	634.4	634.4	634.4	651.7	651.7	651.7

[1]Vit+Min mix provides per kilogram of the diet: Vit. A, 12 000 IU, vit. E (dl-a-tocopheryl acetate) 20 mg, menadione 2.3 mg, Vit. D3, 2 200 ICU, riboflavin 5.5 mg, calcium pantothenate 12 mg, nicotinic acid 50 mg, Choline 250 mg, vit. B12 10 mg, vit. B6 3 mg, thiamine 3 mg, folic acid 1 mg, d-biotin 0.05 mg, Mn 80 mg, Zn 60 mg, Fe 35 mg, Cu 8 mg, Selenium 0.1 mg.
[2]Calcium and phosphorus of the phytase supplemented-diet were adjusted according to phytase equivalent value.

Haematological and biochemical characteristics

Blood samples were collected in heparinized tubes from six birds per treatment at 37 d of age. Plasma was separated by centrifugation at 3 000 rpm for 10 min and stored at −18 °C until analysis. All biochemical traits of blood plasma (total protein, albumin, total cholesterol triglycerides, glucose and ALT) were determined using commercial diagnosing kits (Diamond Diagnostics Company, Egypt) as reported by Attia *et al.* (2009, 2011a and b). Globulin

concentration was calculated as the difference of total protein – albumin. Ten blood samples per treatment were collected to determine haemoglobin, RBC, white blood cells (WBC), different types of leukocytes, haemoglobin concentration, packed cell volume (PCV) and phagocytic activity and index according to Attia *et al.* (2013).

Statistical analyses

Data were processed using the general linear model (GLM) procedure of SAS v9 (SAS Institute Inc., Cary, NC, USA) by a two-way factorial design (four types of feed form by three enzymes treatments and their interaction). Mean difference at $P \leq 0.05$ was tested using Student-Newman-Keuls-test. All percentages were transformed to their corresponding arcsin value before run the analyses.

Results

Carcass traits

The carcass and body organs characteristics of broilers as affected by form of diet and enzyme supplementation are shown in Table 2. The body weight at slaughter was higher ($P<0.01$) in broilers fed pellet in comparison to mash diet. Pellet diets resulted in lower ($P<0.0001$) relative weight of gizzard than mash diets. However, percentage of abdominal fat of broilers fed the 3-3.5 mm pellet diet was higher ($P<0.005$) than that of broilers fed mash and 2-2 mm pellet diet. The percentage of abdominal fat in broilers fed 2-3 mm pellet was not different than that of the other groups. Furthermore, percentage of caecum length of groups fed 3-3.5 mm pellet diet was significantly lower than that of broilers fed the mash diet. The caecum length of birds fed 2-2 and 2-3 mm pellet was between the other two groups.

Table 2
Relative weight of carcass characteristics and body organs of 37 d old broilers as affected feed form and enzymes supplementations

Treatment	Body weight, g	Carcas, g	Dressing, %	TEP, %	IP, %	Gizzard, %	Abdominal fat, %	Caecum length, %
Feed form								
Mash	1780^{b}	1277^{b}	71.8	75.9	24.1	1.40^{a}	0.657^{b}	1.139^{a}
Pellet 2-2 mm	1968^{a}	1429^{a}	72.9	76.7	23.3	1.10^{b}	0.653^{b}	1.028^{ab}
Pellet 2-3 mm	2001^{a}	1463^{a}	73.1	77.0	23.0	1.08^{b}	0.823^{ab}	1.025^{ab}
Pellet 3-3.5 mm	1992^{a}	1481^{a}	74.4	78.4	21.6	1.18^{b}	1.014^{a}	0.977^{b}
Enzymes supplementation								
Control	1 940	1 395	71.9^{b}	75.7^{b}	24.3^{a}	1.19	0.850	1.05
Phytase	1 945	1 416	72.7^{ab}	76.7^{ab}	23.3^{ab}	1.21	0.762	1.02
Multi-enzyme+phytase	1 922	1 427	74.6^{a}	78.6^{a}	21.4^{b}	1.17	0.748	1.06
SEM	30.2	24.6	1.52	1.58	1.58	0.077	0.135	0.059
P-value								
Feed form	0.004	0.0001	ns	ns	ns	0.0001	0.005	0.013
Enzymes suppl.	ns	ns	0.046	0.042	0.042	ns	ns	ns
Interaction	ns	ns	ns	ns	ns	ns	ns	ns

^{a,b,c}Means in the same column followed by different letters are significantly different at $P \leq 0.05$; ns: not significant; SEM: standard error of mean, TEP: total edible parts, IP: inedible parts

The group fed diets supplemented with multi-enzyme+phytase showed greater ($P<0.05$) carcass yield and total edible and lower total inedible parts than the control groups. Groups supplemented with phytase exhibited intermediate values. There was no significant influence of the interaction for all the criteria reported in Table 2.

Meat quality

The chemical and physical characteristics of broiler meat affected by feed form and enzyme supplementation are shown in Table 3. The meat moisture was significantly lower in broilers fed 2-3 mm pellet than that of the groups fed mash diet or 3-3.5 mm pellet. Meat lipid of broilers fed 2-2 and 2-3 mm pellet diets was significantly higher than that of broilers fed mash and 3-3.5 mm pellet diet. However, meat ash was significantly higher in broilers fed 2-3 mm and 3-3.5 mm pellet diet than that of those fed 2-2 mm pellet. There was no effect of feed form on protein as well as on physical characteristics of meat.

Table 3
Chemical composition and physical characteristics of fresh meat of 37 d old broiler as affected feed form and enzymes supplementation

	Chemical composition of meat, %				Physical characteristics of meat	
	Moisture	Protein	Lipid	Ash	Colour	Tenderness
Feed form:						
Mash	68.6[a]	22.5	5.06[b]	3.90[ab]	0.193	2.73
Pellet 2-2mm	66.9[ab]	23.1	6.36[a]	3.60[b]	0.201	2.74
Pellet 2-3mm	65.8[b]	23.8	6.35[a]	4.01[a]	0.214	2.82
Pellet 3-3.5mm	68.3[a]	22.2	5.34[b]	4.20[a]	0.184	2.75
Enzymes supplementation						
Control	68.6[a]	22.1[b]	5.60	3.73	0.188	2.74
Phytase	67.2[b]	22.9[ab]	5.75	4.07	0.208	2.8
Multi-enzyme+phytase	66.3[b]	23.7[a]	5.99	3.98	0.198	2.73
P-value						
Feed form	0.014	ns	0.0002	0.026	ns	ns
Enzymes	0.002	0.005	ns	ns	ns	ns
Interaction	0.0001	0.019	0.0001	ns	ns	0.009
Standard error of mean	1.19	0.99	0.40	0.42	0.015	0.065

[a,b,c]Means in the same column followed by different letters are significantly different at $P\leq0.05$, ns: not significant

Phytase+multi-enzyme resulted in significantly lower meat moisture than the unsupplemented control but, phytase+multi-enzyme significantly induced higher meat protein than unsupplemented diet. Phytase supplemented group showed intermediate meat protein. There was no significant influence of enzyme supplementations on meat lipid and ash and meat physical characteristics.

A significant interaction of feed form×enzyme supplementations was shown for meat moisture, protein and lipid percentages and meat tenderness. In the control group higher levels of meat moisture were recorded with mash and 3-3.5 mm pellet diet. When the broilers' diets were supplemented with phytase, there were similar moisture contents for mash, 2-3 mm and 3-3.5 mm pellet diet but higher values were observed for the other diet. The groups fed phytase and multi-enzymes showed no differences in meat moisture. The

level of meat protein was lower in the control groups when broilers fed mash and 3-3.5 mm pellet diet, while no difference was observed for 2-2 diet. With 2-3 mm pellet diet, a lower value of meat protein was recorded with phytase supplementation. The amount of lipids was lower in the control group than in the phytase or multi-enzyme groups when mash and 3-3.5 mm diets were fed. The opposite occurred with 2-2 mm pellet diet. For meat tenderness the group fed 2-3 mm pellet diet+phytase showed the highest value, while no differences were observed among the other groups.

Biochemical constituents of plasma

The plasma biochemical constituents of broilers are shown in Table 4. Albumin/globulin ratio was higher ($P<0.05$) in broilers fed 3-3.5 mm pellet diet than in those fed 2-3 mm pellet. Cholesterol was higher ($P<0.01$) in 2-3 mm pellet diet than that in the others. Broilers fed 2-2 mm and 2-3 mm pellet diet showed higher ($P<0.05$) plasma glucose than those fed mash diets. Plasma ALT was higher ($P<0.01$) in broilers fed 2-2 mm pellet diet than only that in those fed 3-3.5 mm pellet.

Table 4

Biochemical constituents of blood serum of 37-d old broilers as affected feed form and enzymes supplementation

Treatment	TP g/100ml	Alb g/100ml	Alb/Glob	Glu mg/dl	Trigl mg/dl	Chol mg/100ml	ALT U/l	AST/ALT
Feed form								
Mash	4.68	2.63	1.32^{ab}	77.2^{b}	187	202^{b}	69.9^{ab}	0.895
Pellet 2-2 mm	4.97	2.69	1.24^{ab}	82.1^{a}	190	190^{b}	72.5^{a}	0.862
Pellet 2-3 mm	5.06	2.54	1.05^{b}	81.7^{a}	189	208^{a}	70.5^{ab}	0.878
Pellet 3-3.5 mm	4.89	2.79	1.54^{a}	80.3^{ab}	191	204^{b}	68.3^{b}	0.929
Enzymes								
Control	4.70^{b}	2.62	1.35	76.4^{b}	186^{b}	206^{a}	66.9	0.899
Phytase	5.03^{a}	2.66	1.20	82.5^{a}	191^{a}	192^{b}	70.8	0.884
Multi-enzyme+phytase	4.98^{a}	2.72	1.31	82.2^{a}	191^{a}	206^{a}	70.5	0.889
P-value								
Feed form	ns	ns	0.044	0.041	ns	0.003	0.003	ns
Enzymes	0.048	ns	ns	0.003	0.025	0.002	ns	ns
Interaction	ns	0.009	ns	ns	ns	0.01	0.0001	0.007
Standard error of mean	0.19	0.14	0.20	2.25	2.48	1.77	1.23	0.03

^{a,b,c}Means in the same column followed by different letters are significantly different at $P \leq 0.05$, ns: not significant, TP: total protein, Alb: albumin, Glob: globulin, Glu: glucose, Trigl: triglycerides, Chol: cholesterol

Enzyme supplementations resulted in higher total protein ($P<0.05$), plasma glucose ($P<0.01$) and triglycerides ($P<0.05$) than the control group but phytase alone decreased ($P<0.01$) cholesterol compared to the other groups.

A significant interaction diet form x enzymes supplementation was shown only on plasma albumin, cholesterol, ALT and ALT/AST ratio. The level of albumin was quite similar among groups when 2-3 and 3-3.5 mm pellet diets were fed but with 2-2 mm pellet diet the use of multi-enzyme+phytase increased the albumin than the other groups. The cholesterol level was almost constant (an average 206 mg/100 ml) in the control groups and had a similar

trend for phytase and multyenzime + phytase group when pellet diets were fed; however with mash diet, phytase reduced the cholesterol level than the other groups. The ALT levels were higher for mash and 3-3.5 mm pellet diet when broilers fed multi-enzyme+phytase, while the opposite happened for control and phytase groups. The AST/ALT ratio of broilers fed mash and 3-3.5 mm pellet diets was lower with multi-enzyme+phytase supplementation while the opposite occurred for 2-2 and 2-3 mm pellet diets.

Blood haematology and immune indices:

The blood profiles of broilers are shown in Table 5. Feeding 2-3 mm pellet diet significantly decreased haemoglobin, PCV, WBC and phagocytic activity and index compared to mash diet whereas an increasing pellet diameter to 3-3.5 mm resulted in similar haemoglobin and PCV in comparison to mash diet but in a higher value of RBC than mash and 2-2 mm pellet diet. Increasing pellet diameter to 3-3.5 mm significantly increased WBC and decreased PI in comparison to 2-3 mm pellet diet. Broilers fed 2-3 mm and 3-3.5 mm pellet had higher ($P<0.01$) levels of lymphocyte and monocytes and lower ($P<0.01$) values of heterophils and H/L ratio than those on mash and 2-2 mm pellet diet. Increasing pellet diameter from 2-3 mm to 3-3.5 mm significantly decreased lymphocytes but increased heterophil level and H/L ratio.

Phytase supplementation significantly increased RBC and PA but decreased PI compared to the control group. Phytase+multi-enzyme significantly increased RBC and WBC but decreased PI in comparison to the control group. Lymphocytes were significantly decreased in phytase supplemented group compared to other groups while monocytes increased. Multi-enzymes+phytase increased lymphocyte percentage but decreased monocytes in comparison to the phytase administered alone.

A significant interaction between form of diet and enzyme supplementation was shown on PCV, RBC, WBC, PI, lymphocytes, monocytes, heterophils and H/L ratio. The PCV value was almost constant with mash diet; progressively increased from control to multi-enzyme+phytase with 3-3.5 mm pellet diet. The level of RBC was similar in control, phytase and multi-enzyme+phytase groups when mash diet was administered; with 2-3 mm and 3-3.5 mm pellet diets the RBC content progressively increased from mash to multi-enzyme+phytase diet while with 2-2 mm diet the RBC content measured in multi-enzyme+phytase groups was lower than that observed in the other groups. The great difference in WBC contents was observed in phytase supplemented groups as the cells were minimized to 2-2 mm and maximized to 3-3.5 mm diets. The phagocytic index showed a similar trend for phytase and multi-enzyme+phytase groups with higher values with mash and 2-3 mm pellet diets; the unsupplemented groups had lower values of PI with 2-3 mm and 3-3.5 mm pellet diets. The percentage of lymphocytes was similar among groups when 2-3 mm pellet diet was fed and was, respectively, minimized and maximized due to addition of phytase when mash and 2-2 mm or 3-3.5 mm pellet diets were fed. While for mash and 2-2 mm pellet diets the percentage of monocytes was slightly affected by different supplementations, the use of phytase gave higher levels of monocytes with 2-3 mm and 3-3.5 mm pellet diets. Higher values of heterophils were measured with phytase in mash and 2-2 mm pellet diets. The supplementation of multi-enzyme+phytase maximized the H/L ratio in mash and 2-2 mm pellet diets; control and phytase groups gave higher values of H/L ratio than multi-enzyme+phytase when 3-3.5 mm pellet diet was fed to broilers.

Table 5
Blood haematological and immune indices of 37 d old broiler as affected feed form and enzymes supplementation

Treatment	Hgb, g/100ml	PCV, ml/100ml	RBC, millions/ mm^3	WBC, millions/mm^3	PA	PI	L,%	Mon,%	H,%	H/L
Feed form:										
Mash	9.73[a]	29.3[a]	1.44[b]	24.3[a]	17.6[a]	1.81[a]	36.2[c]	4.47[b]	49.2[a]	1.36[a]
Pellet 2-2 mm	9.67[a]	28.8[ab]	1.52[b]	24.5[a]	17.9[a]	1.54[c]	36.4[c]	4.37[b]	49.5[a]	1.37[a]
Pellet 2-3 mm	9.00[b]	27.6[b]	1.54[ab]	22.1[b]	15.4[b]	1.68[b]	39.9[a]	6.17[a]	45.0[c]	1.13[c]
Pellet 3-3.5 mm	9.30[ab]	28.4[ab]	1.62[a]	24.90[a]	15.0[b]	1.51[c]	38.0[b]	6.57[a]	46.5[b]	1.23[b]
Enzymes supplementation										
Control	9.23	27.9	1.46[b]	23.3[b]	16.0[b]	1.78[a]	37.8[a]	5.13[b]	47.8	1.27
Phytase	9.6	28.9	1.60[a]	23.8[b]	17.0[a]	1.50[c]	37.1[b]	6.10[a]	47.5	1.26
Multi-enzyme+phytase	9.45	28.8	1.54[a]	24.8[a]	16.5[ab]	1.63[b]	37.9[a]	4.95[b]	47.4	1.29
P-value										
Feed form	0.022	0.044	0.0011	0.0001	0.0001	0.0001	0.001	0.001	0.001	0.0001
Enzymes	ns	ns	0.0017	0.0001	0.009	0.0001	0.018	0.001	ns	ns
Interaction	ns	0.009	0.0001	0.0001	ns	0.0001	0.0001	0.0001	0.0001	0.0001
Standard error of mean	0.32	0.76	0.05	0.41	0.44	0.06	0.44	0.34	0.69	0.03

[a,b,c]Means in the same column followed by different letters are significantly different at ($P \leq 0.05$), Hgb: haemoglobin, L: lymphocytes, Mon: monocytes, H: heterophils, ns: not significant

Discussion

Effect of feed form

The higher body weight at slaughter recorded with pellet diets was in line with other authors (Munt *et al.* 1995, Preston *et al.* 2000, Ghazi *et al.* 2012) who reported that the higher body weight was tied to a higher feed intake of broilers. The higher body weight also induced a higher weight of carcass with pellet diets. The decrease of gizzard percentage when pellet diet was fed is in line with the findings of Nyr *et al.* (1995) and Engberg *et al.* (2002). Also, according to Svihus *et al.* (2004) no effect of pellet size was recorded on gizzard percentage. The gizzard is a muscular organ that reduces particle size of ingested feeds and mixed them with digestive enzymes (Duke 1986). However, when pellet diets are fed, the particle size is tied to the particle size distribution after pellet dissolution in the crop: as a consequence, when the ingredients are finely grinded before pelleting, there is a lack of difference in particle size distribution after pelleting (Svihus *et al.* 2004). Regarding the other tracts of the digestive system, only the length of the caecum was reduced in comparison to the mash diet when birds fed 3-3.5 mm pellet diet and, even if not significant, the caecum length in birds fed the other two pellet diets was lower than that in the mash diet. This reduction is not easy to explain and, as limited to a specific gut compartment, it is probably tied to a redundancy of the digestive action in this tract. Also Amerah (2008) found a decrease of caecum length in poultry fed pellet diet in comparison to that fed a mash diet. The percentage of abdominal fat was significantly increased in the group fed 3-3.5 mm pellet diets in comparison to that in the mash group even if there is a tendency in abdominal fat percentage increase as the size of pellet increased. Sarvestani *et al.* (2006) also found an increase in abdominal fat percentage when broilers fed pellet instead of mash diet. However, the changes in abdominal fat percentages were not tied to a change in fat metabolism as the level of triglycerides was independent from feed form and cholesterol level modification were not in line with the changes of abdominal fat percentages.

The increase in abdominal fat percentage and in fat content of meat from broilers fed pellet diet 2-2 mm and 2-3 mm could be attributed to the decrease in energy expenditure for mechanical digestion and grinding in the gizzard. According to our results, Amerah *et al.* (2007c) reported that broilers fed pellet showed a decrease in the relative length of all parts of the digestive tract.

In literature, blood biochemical and haematological constituents of poultry were affected by genetic and environment factors (Attia *et al.* 2001, 2011a, b). Feed is one environmental factor that had greater influence on lipid and cholesterol metabolism. The present results demonstrated that feed form affected biochemical and haematological constituents of blood of broilers. The increase in plasma glucose and cholesterol in group fed 2-3 mm pellet diameter diet was concurred with increasing meat lipid.

Andi *et al.* (2011) found that difference in AST level between birds fed pelleted and mash diets were insignificant. In contrary, Corzo *et al.* (2012) reported that blood glucose was insignificantly affected by feed form, but total protein was significantly higher in broilers fed pelleted diet vs. those fed mash diet while albumin was found to be less in broilers fed mash than that in those fed pellet diet.

There were also negative changes in blood haematological traits (haemoglobin and PCV) with increasing pellet diameter to 2-3 mm although further increase to 3-3.5 mm restored

blood haematological traits to the level of that of mash and 2-2 mm pellet diameter diet, indicating an improvement in the haematological traits. Similar trend was shown in WBC.

Our results showed that form of feeds significantly affected blood haematological traits and this depends on pellet diameter.

Effect of enzyme supplementation

An additive effect of multi-enzyme over phytase was shown in relative weight of dressing and total edible parts (3.8 vs. 1.22 %) and this concurred with a greater meat protein percentage (10.8 vs. 5.6 %) while decreasing meat moisture (4.4 vs. 2.8 %). This indicated that multi-enzymes showed synergic effects with phytase that resulted in an improvement in meat quality traits. These results are similar to those reported by Abd-Elsamee (2002), Attia *et al.* (2003a, b) and Salem *et al.* (2003) who concluded that the improvement in carcass yield due to enzyme supplementation is a reflection of the increase in nutrient availability for tissue growth.

Both phytase without or with multi-enzyme supplementations showed similar positive effects on total protein (+7 and +6 %, respectively), plasma glucose (+8.1 and +7.7 %, respectively), triglycerides (+2.22 and +2.36 %, respectively) and RBC (+9.6 and +5.5 %, respectively). These changes in blood metabolism could be attributed to the increase in nutrient digestibility (Attia *et al.* 2003a,b, 2008) and suggests that phytase administered alone was sufficient to improve nutrient digestibility of broilers. Phytase alone decreased cholesterol by 7 % and increased phagocytes activity by 6.1 %.

The lack of significant effect of enzymes on blood albumin, globulin, haemoglobin, PCV, basophils, heterophils, H/L ratio and enzymes (AST and ALT) suggests that phytase or Avizyme had no adverse effect on plasma constituents and liver activity. In addition, as AST is a mitochondrial enzyme also found in peripheral tissues as muscles (Moniello *et al.* 2005, Bovera *et al.* 2007), also the muscular tropism seems to be unaffected by dietary treatments. These results are in general agreement with the results reported by Attia *et al.* (2001, 2003a,b), Qota *et al.* (2002) and El-Ghamry *et al.* (2005). They concluded that enzyme supplementation to broilers and ducks diets had no significant effect on plasma constituents. In addition, Ibrahim and Saleh (2005), Salem *et al.* (2008) and Elmenawey *et al.* (2010) reported that enzyme cocktail supplementation had no significant effect on plasma ALT concentration.

Effect of the interaction

There are several interactions between form of feed and enzyme supplementation on biochemical constituents of plasma (e.g. albumin, cholesterol, ALT and ALT/AST ratio); haematological traits (PCV, RBC, WBC, PI, lymphocyte, monocytes, heterophils, H/Lratio) and meat quality (moisture, fat, protein and meat tenderness). The effects were in general different for the different evaluated criteria and it is not easy to have a general conclusion about this. However, it is clear that the effect of enzyme depends on type of diet (pellet vs. mash). Phytase+multi-enzyme showed synergic effects on blood contents and meat quality of broilers based on pellet diameter as 2-3 mm pellet diameter diet showed greater effect than 2-2 mm pellet diet while increasing pellet diameter to 3-3.5 mm resulted in most cases in diminishing of response. An increase in welfare was shown when phytase+multi-

enzyme was added to 2-3 mm and 3-3.5 mm pellet diameter diets. However, addition of phytase+multi-enzyme increased stress on broilers fed mash and 2-2 mm pellet diameter diet as indicated by increased (H/L) ratio may be due to boredom effect. Multi-enzymes+phytase showed greater effect (synergetic) on blood parameters and meat quality of broilers fed diet with 3-3.5 mm pellet diameter than 2-2 mm and 2-3 mm pellet diets. However, the lack of significant interaction between form of feed and enzyme supplementations on carcass characteristics and body organs and some blood parameters and meat quality indicated that either multi-enzyme+phytase or phytase alone supplementation to mash or pellet feeds is essential and the influence of enzymes is independent of type of feeds.

In general, pellet form of feed supplemented with multi-enzyme+phytase or phytase alone improved blood parameters and meat quality and multi-enzyme+phytase resulted in the highest percentages of dressing and total edible parts of broilers.

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